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Prognostic value of stromal cell-derived factor 1 expression in patients with gastric cancer after surgical resection

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Key words

Gastric cancer, overall survival, predictive nomogram, prognostic biomarker, stromal cell-derived factor 1

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Aberrant chemokine stromal cell-derived factor 1 (SDF-1) expression has been shown to be involved in the development and progression of various malignancies. Our present study aims to investigate the clinical and prognostic value of SDF-1 expression and improve risk stratification in patients with gastric cancer. Peritumoral and intratumoral SDF-1 levels were assessed in 220 retrospectively enrolled gastric cancer patients, and their relations with clinicopathological features and clinical outcomes were evaluated. A predictive nomogram was created to refine risk stratification for overall survival of gastric cancer patients. Compared with peritumor tissues, tumor tissues showed decreased SDF-1 expression levels according to TNM stage progression in gastric cancer specimens. Peritumoral SDF-1 expression correlated positively with tumor invasion depth and lymph node metastasis, whereas intratumoral SDF-1 expression associated negatively with tumor size, tumor differentiation, tumor invasion depth, lymph node metastasis, and clinical TNM stage. Moreover, both low peritumoral SDF-1 expression and high intratumoral SDF-1 expression indicated favorable overall survival, and SDF-1 risk derived from the peritumoral/intratumoral SDF-1 expression signature could stratify prognosis of patients with gastric cancer. After backward elimination, SDF-1 risk was identified as an independent prognostic factor for survival. Finally, a predictive nomogram was generated with identified independent prognosticators to assess patient survival at 3 and 5 years following surgery. Conclusively, SDF-1 risk, an identified independent prognostic factor, could be developed into a nomogram with tumor invasion depth, lymph node involvement, and distant metastasis to refine predictive accuracy for survival in patients with gastric cancer after surgical resection.

Although the incidence and mortality of gastric cancer have declined over the past decades, it continues to be the fourth most common malignant neoplasia worldwide and ranked as the second leading cause of cancer-associated mortality.[1] Surgical resection is the only possible curative method for gastric cancer, especially for patients in the early stage of the disease.[2] However, many patients are diagnosed at an advanced stage due to atypical symptoms in the early stages. For patients with advanced-stage gastric cancer, the prognosis is dismal due to the high rate of metastasis or postsurgical relapse.[3] Therefore, there is growing interest in gaining a better understanding of the molecular and cellular processes in gastric cancer to develop more reliable biomarkers to predict outcomes of patients with particularly aggressive disease for optimal medical treatment. However, the mechanisms underlying the molecular and cellular behaviors remain largely unknown and need to be further established.

Chemokines are small 8–12-kDa peptides that regulate chemotaxis.[4] Stromal cell-derived factor 1 (SDF-1), also known as CXC-chemokine 12 (CXCL12), is a small (68 amino acids, 8 kDa) chemokine that has been identified as the ligand for cell-surface CXC-chemokine receptor 4 (CXCR4) and receptor 7 (CXCR7). First identified as a growth factor for B cell progenitor cells, SDF-1 is essential for lymphocyte trafficking and maintenance of immune balance.[5] SDF-1 exerts its function by interacting with its physiological receptor, activating the downstream protein kinase B/MAPK pathway, leading to

alteration of gene expression, actin polymerization, cell skeleton rearrangement, and cell migration.[6]

A growing number of studies have identified that SDF-1 is also expressed in many human tumor cells, such as ovarian cancer,[7] breast cancer,[8] glioblastoma,[9] pancreatic cancer,[10] prostate cancer,[11] and thyroid cancer,[12] and acts in an autocrine or paracrine manner. The constitutively activated SDF-1/CXCR4 axis has been shown to play a crucial role in promoting tumor growth through paracrine and/or autocrine stimulation of tumor cells, promoting tumor invasion and metastasis by stimulating expression of MMPs, and trafficking tumor cells to target organs or tissues along ligand gradients.[6] Moreover, tumor cells may use CXCR4 to access the SDF-1-rich niche microenvironment to favor their survival and resistance to chemotherapy.[13,14] SDF-1 can also upregulate the expression of vascular endothelial growth factor and recruit vasculature-supporting bone marrow-derived progenitor cells to the tumor site to promote angiogenesis and vasculogenesis.[15,16] Tumor cells may take advantage of these chemokine-mediated mechanisms during the process of progression and organ selective metastasis. Recent studies have identified that CXCR4, the SDF-1 receptor, was overexpressed in gastric cancer cells and correlated with tumor progression and metastasis.[17,18] However, the role of SDF-1 in gastric cancer is still controversial because reports have shown that SDF-1 mRNA and protein levels in gastric cancer were reduced compared with non-tumor tissues.[19,20] Thus, studies aimed to elucidate the prognostic values of SDF-1 expression in patients with gastric cancer were urgently needed.

At present, the TNM staging system provides the major prognostic variables used in clinical management of patients with gastric cancer. However, these clinicopathological parameters do not provide complete prognostic information. For example, patients with similar disease morphologies may display different biological phenotypes and prognoses. Some patients in the early tumor stage may progress rapidly, whereas others in the advanced stage may stay stable for years. This is partly owing to tumor heterogeneity.[21] The SDF-1 expression signature in patients with gastric cancer may be a potential mechanism underlying tumor heterogeneity. Therefore, incorporation of the prognostic information derived from the SDF-1 expression signature with the traditional TNM staging system may refine a risk stratification system for clinical outcomes and provide more specific treatment advice.

In this study, we investigated the expression of SDF-1 in patients with gastric cancer, and explored its relation with clinicopathological factors and clinical outcomes. A predictive nomogram was generated to evaluate the risk for overall survival (OS) of gastric cancer patients. The prognostic accuracy was examined by calibration curve and time-dependent receiver operating characteristic (ROC) curve analysis.

Materials and Methods

Patients and specimens. A total of 180 consecutive gastric cancer patients who received standard gastrectomy with D2 lymph node resection from the same surgical team in Zhongshan Hospital of Fudan University (Shanghai, China) between May 2002 and April 2006 were enrolled in the study. We retrospectively collected the baseline demographic and clinicopathological factors of these patients, including age, gender, tumor location, tumor size, tumor differentiation, Lauren classification, and tumor stage. Tumor stage and tumor differentiation were reassessed by two independent gastroenterology pathologists according to the 2010 International Union Against Cancer TNM classification system. The median age of this cohort was 63 years (range, 32–83), of which 68.9% were men. Patients with intestinal type disease made up 66.1% of the group, and the remainder had diffuse type. Lymph node involvement was evident in 68.9% of patients; six patients (3.3%) had resectable synchronous single liver metastases at the time of surgery. All patients were followed up until July 2012, with a median follow-up time of 59 months. Overall survival was defined as the time between the dates of surgery and death or last visit. An additional 40 patients were recruited between January and April, 2013, and their resected samples were subjected to RNA extraction for quantitative RT-PCR and ELISA examination. The use of human specimens was approved by the Clinical Research Ethics Committee of Zhongshan Hospital with informed consent from each patient. No patients received any preoperative anticancer treatment.

RNA extraction and RT-PCR. Total RNA from gastric cancer samples was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Two micrograms of total RNA was converted to cDNA using a Reverse Transcription System kit (Takara Bio Inc., Otsu, Japan). Real-time PCR was carried out using a StepOne Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) and SYBR Green PCR kit (Applied Takara). The primer sequences used in this study were: GTC AAG CAT CTC AAA ATT CTC AAC AC (sense) and CAC TTT AGC TTC GGG TCA ATG C (antisense), for SDF-1; and CAT GAG AAG TAT GAC AAC AGC CT (sense) and AGT CCT TCC ACG ATA CCA AAG T (antisense), for GAPDH.[20]

Enzyme-linked immunosorbent assay. Frozen gastric tissues were ground to a powder in liquid nitrogen and stored at −80°C. For protein preparation, 250 μL lysis buffer (1% CHAPS, 50 mM DTT, 10 mM EDTA, 1 mM PMSF, 1 μg/mL pepstatin A, and 1 μg/mL leupeptin in PBS [pH 7.2]) were added to 0.1 g tissue powder and mixed vigorously. Ultrasonication (100 W for 5 s, repeated 5–7 times) was used to further break the mixture into small particles. Finally, insoluble substances were removed by centrifugation (20 000*g* for 50 min at 4°C) and the supernatants harvested. The protein concentration was determined using the Bradford method. The expression of SDF-1 levels was detected by ELISA according to the manufacturer's manual, as described previously.[22] Experiments were carried out in triplicate. The ELISA kits were obtained from R&D Systems (Minneapolis, MN, USA).

Immunohistochemistry and evaluation. The construction of tissue microarray and the immunohistochemistry (IHC) protocols were as described previously.[23] Collectively, two cores were taken from the center area of each representative tumor tissue and from normal gastric tissue adjacent to the invasive tumor front within a distance of 10 mm to construct tissue microarray slides. Cylinders from the two different areas, intratumoral and peritumoral, were obtained for each patient. Then, tissue microarray sections with 180 pairs of tumors and matched peritumoral samples were constructed. The primary antibody was mouse mAb against SDF-1 (10 μg/mL MAB350; R&D Systems). The density of the positive staining was evaluated by a computerized imaging system composed of an Olympus CCD camera (Olympus Corporation, Tokyo, Japan) connected to a Nikon Eclipse Ti-S microscope (Nikon Instruments Inc., Melville, NY, USA). The IHC sections were scanned at low power (×100) magnification by NIS-Elements F3.2 software (Nikon) to identify the five areas with the greatest positive staining. Then the mean density was estimated at

high power (×200) magnification from these five areas per case. Identical settings were used for each photograph. The density of positive staining was counted by Image-Pro plus version 6.0 software (Media Cybernetics, Bethesda, MD, USA) by two pathologists who were blind to the characteristics of the patients. Integrated optical density of all the positive staining in each photograph was measured, and its ratio to total area of each photograph was calculated as relative density. The cut-off value for the definition of high/low expression subgroups was the median density value; 77.75 was defined as the cut-off for peritumor tissues and 34.38 was defined as the cut-off for tumor tissues.

Statistical analysis. Statistical analysis was carried out with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and R software (http://www.r-project.org/). Differences between scatter plots for density of IHC staining were determined by the non-parametric Mann–Whitney *U*-test. Pearson's chi-square-test or Fisher's exact test was used to compare qualitative variables. Kaplan–Meier analysis was used to determine survival. The log–rank test was used to compare patients' survival between subgroups; the stepwise Cox regression model was used to carry out the multivariate analysis. Only factors demonstrating an association with OS ($P < 0.010$) were included in the multivariate analysis. Numbers at risk were calculated for the beginning of each time period. Nomograms were created by R software using the "rms" package. A calibration plot was generated to examine the performance characteristics of nomograms. The time-dependent ROC curve analysis and bootstrap-corrected concordance index (C-index) were used to compare the discrimination power for OS between different models. All statistical analyses were two-sided and $P < 0.05$ was considered statistically significant. Results are reported according to Reporting Recommendations for Tumor Marker Prognostic Studies guidelines.[24]

Results

Frequently decreased expression levels of SDF-1 in gastric cancer tissues. To clarify the underlying role of SDF-1 in gastric cancer, we first examined the expression levels of SDF-1

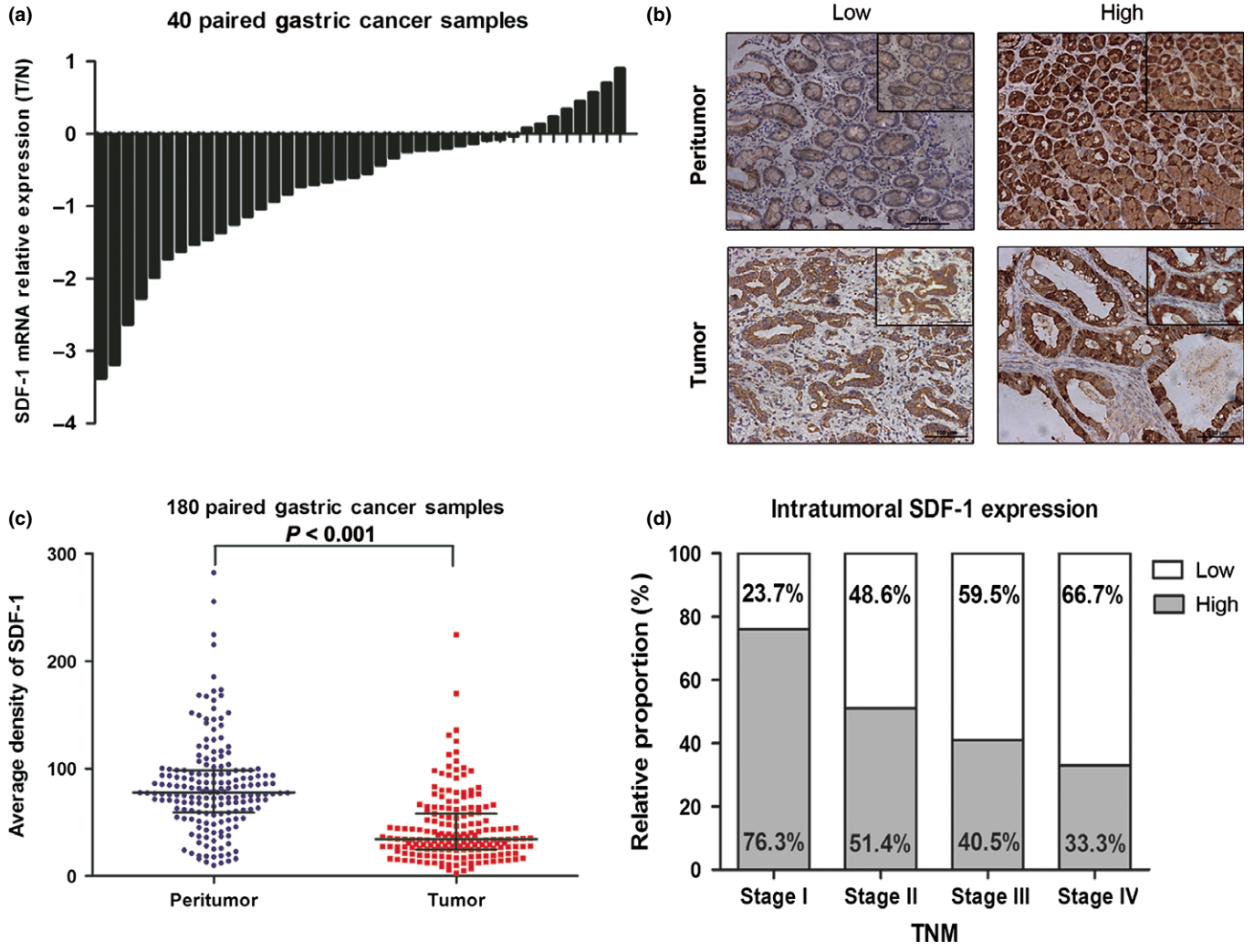

Fig. 1. Frequently reduced stromal cell-derived factor 1 (SDF-1) expression in gastric cancer tissues. (a) Expression levels of SDF-1 mRNA in 40 paired gastric cancer and adjacent non-tumor tissues were evaluated by real-time PCR. (b) Representative immunohistochemical staining of SDF-1 in gastric cancer and peritumoral tissues. Original magnification (200×, top right panels) and higher magnification (400×) are shown. (c) Relative immunohistochemical staining of SDF-1 in paired gastric cancer tissue samples ($n = 180$). The SDF-1 expression level was significantly down-regulated in tumors compared with corresponding adjacent non-tumor gastric tissues. (d) Relative portion of intratumoral SDF-1 expression according to tumor stage. Low SDF-1 expression portion was elevated as the tumor stage progressed. The bars indicate the median value with interquartile range.

Table 1. Relation between peritumoral stromal cell-derived factor 1 (SDF-1) expression or intratumoral SDF-1 expression and clinical characteristics in patients with gastric cancer (n = 180)

Factors	Patients		Peritumoral SDF-1 expression			Intratumoral SDF-1 expression			Peritumoral/intratumoral SDF-1 expression				
	No.	%	Low	High	*P*-value	Low	High	*P*-value	Low/High	High/High	Low/Low	High/Low	*P*-value
All patients	180	100	90	90		90	90		42	48	48	42	
Age, years†													
≤63	100	55.6	48	52	0.549	47	53	0.368	23	30	25	22	0.716
>63	80	44.4	42	38		43	37		19	18	23	20	
Gender													
Female	56	31.1	30	26	0.520	29	27	0.747	13	14	17	12	0.890
Male	124	68.9	60	64		61	63		29	34	31	30	
Localization													
Proximal	30	16.7	11	19	0.091	16	14	0.279	4	10	7	9	0.118
Middle	58	32.2	35	23		24	34		17	17	18	6	
Distal	92	51.1	44	48		50	42		21	21	23	27	
Tumor size†													
<4 cm	93	51.7	42	51	0.179	38	55	**0.011**	23	32	19	19	**0.046**
≥4 cm	87	48.3	48	39		52	35		19	16	29	23	
Differentiation													
Well	6	3.3	4	2	0.682	3	3	**0.030**	2	1	2	1	0.220
Moderate	67	37.2	34	33		25	42		21	21	13	12	
Poor	107	59.5	52	55		62	45		19	26	33	29	
Lauren classification													
Intestinal type	119	66.1	56	63	0.270	57	62	0.431	28	34	28	29	0.585
Diffuse type	61	33.9	34	27		33	28		14	14	20	13	
T classification													
T1	32	17.8	19	13	**0.041**	8	24	**0.007**	13	11	6	2	**0.010**
T2	15	8.3	9	6		6	9		5	4	4	2	
T3	11	6.1	9	2		5	6		5	1	4	1	
T4	122	67.8	53	69		71	51		19	32	34	37	
N classification													
N0	56	31.1	33	23	**0.036**	18	38	**0.008**	21	17	12	6	**0.006**
N1	43	23.9	20	23		22	21		7	14	13	9	
N2	28	15.6	18	10		18	10		7	3	11	7	
N3	53	29.4	19	34		32	21		7	14	12	20	
Distant metastasis													
No	174	96.7	87	87	1.000	86	88	0.682	41	47	46	40	0.868
Yes	6	3.3	3	3		4	2		1	1	2	2	

Table 1 (continued)

Factors	Patients		Peritumoral SDF-1 expression			Intratumoral SDF-1 expression			Peritumoral/intratumoral SDF-1 expression				
	No.	%	Low	High	*P*-value	Low	High	*P*-value	Low/High	High/High	Low/Low	High/Low	*P*-value
TNM stage													
I	38	21.1	22	16	0.259	9	29	**0.002**	15	14	7	2	**0.019**
II	35	19.5	21	14		17	18		10	8	11	6	
III	101	56.1	44	57		60	41		16	25	28	32	
IV	6	3.3	3	3		4	2		1	1	2	2	
ACT													
No	69	38.3	39	30	0.168	29	40	0.092	21	19	18	11	0.166
Yes	111	61.7	51	60		61	50		21	29	30	31	

ACT, adjuvant chemotherapy; N, lymph node involvement; T, tumor invasion depth. †Split at median. Values in bold indicate significance.

mRNA in 40 paired gastric cancer samples using quantitative real-time PCR. We found that SDF-1 expression was significantly decreased in tumor tissues compared with matched adjacent non-tumor gastric mucosa for 80% of the gastric samples (Fig. 1a). The protein levels of SDF-1 expression detected by ELISA from tumor tissues were also lower than expression from peritumoral tissues for 87.5% of gastric samples (Fig. S1a), and showed concordance with mRNA levels ($r = 0.78$, $P < 0.001$; Fig. S1b). We then carried out IHC analyses of SDF-1 expression using a gastric cancer tissue microarray containing 180 paired gastric cancer samples. The IHC staining intensity varied greatly in gastric cancer tissues and matched non-tumor tissues (Fig. 1b). The staining intensity of SDF-1 protein in the peritumor group was stronger than that observed in the tumor group (Fig. 1c). High intratumoral SDF-1 expression was more easily seen in patients with early stage tumor (Fig. 1d), whereas peritumoral SDF-1 expression did not show such a phenomenon.

Associations between SDF-1 expression and clinicopathological factors. The relationship between SDF-1 expression and clinicopathological factors are shown in Table 1. Peritumoral SDF-1 expression and intratumoral SDF-1 expression were positively and negatively correlated with T classification ($P = 0.041$ and $P = 0.007$, respectively) and N classification ($P = 0.036$ and $P = 0.008$, respectively). Moreover, intratumoral SDF-1 expression was also negatively associated with tumor size ($P = 0.011$), tumor differentiation ($P = 0.030$), and TNM stage ($P = 0.002$). Combined analysis of peritumoral and intratumoral SDF-1 signatures showed significant correlations with tumor size ($P = 0.046$), T classification ($P = 0.010$), N classification ($P = 0.006$), and TNM stage ($P = 0.019$). These data suggested the significance of SDF-1 expression in tumor biological phenotypes.

Associations of SDF-1 expression and clinical outcomes. To further explore the prognostic significance of SDF-1 expression and clinical outcomes, Kaplan–Meier analysis was used to determine OS and the log–rank test was used to compare differences between subgroups. Using their respective median values as the cut-off for high and low expression, high SDF-1 in peritumor tissue was associated with reduced OS ($P = 0.0034$; Fig. 2a), whereas high SDF-1 in tumor tissue was correlated with elevated OS ($P < 0.0001$; Fig. 2b). We then compared low and high expression of SDF-1 by tumor invasion depth (Fig. S2). Although the SDF-1 expression had no significant correlation with OS in patients with T1 to T3, we found peritumor and intratumoral SDF-1 expression negatively and positively correlated with OS in patients with T4 disease ($P = 0.0147$ and $P = 0.0008$, respectively). To further discriminate patients with different prognoses, we carried out a combined analysis of peritumoral and intratumoral SDF-1 expression (Fig. 2c). Significant differences in OS ($P < 0.0001$) were found among the four groups. In the peritumoral high/intratumoral high group and peritumoral low/intratumoral low group, the influence of intratumoral SDF-1, low or high, on prognosis was probably counteracted by simultaneously low or high peritumoral SDF-1 expression, and vice versa. Therefore, irrespective of the absolute intensity of peritumoral and intratumoral SDF-1 expression, the two groups had similar data for survival (hazard ratio = 0.701; 95% confidence interval, 0.397–1.238; $P = 0.221$). Based on these results, we classified patients into three risk groups (Fig. 2d) according to their peritumoral/intratumoral SDF-1 expression signature: low risk group, peritumoral low/intratumoral high ($n = 42$); intermediate risk group, peritumoral high/intratumoral high and pe-

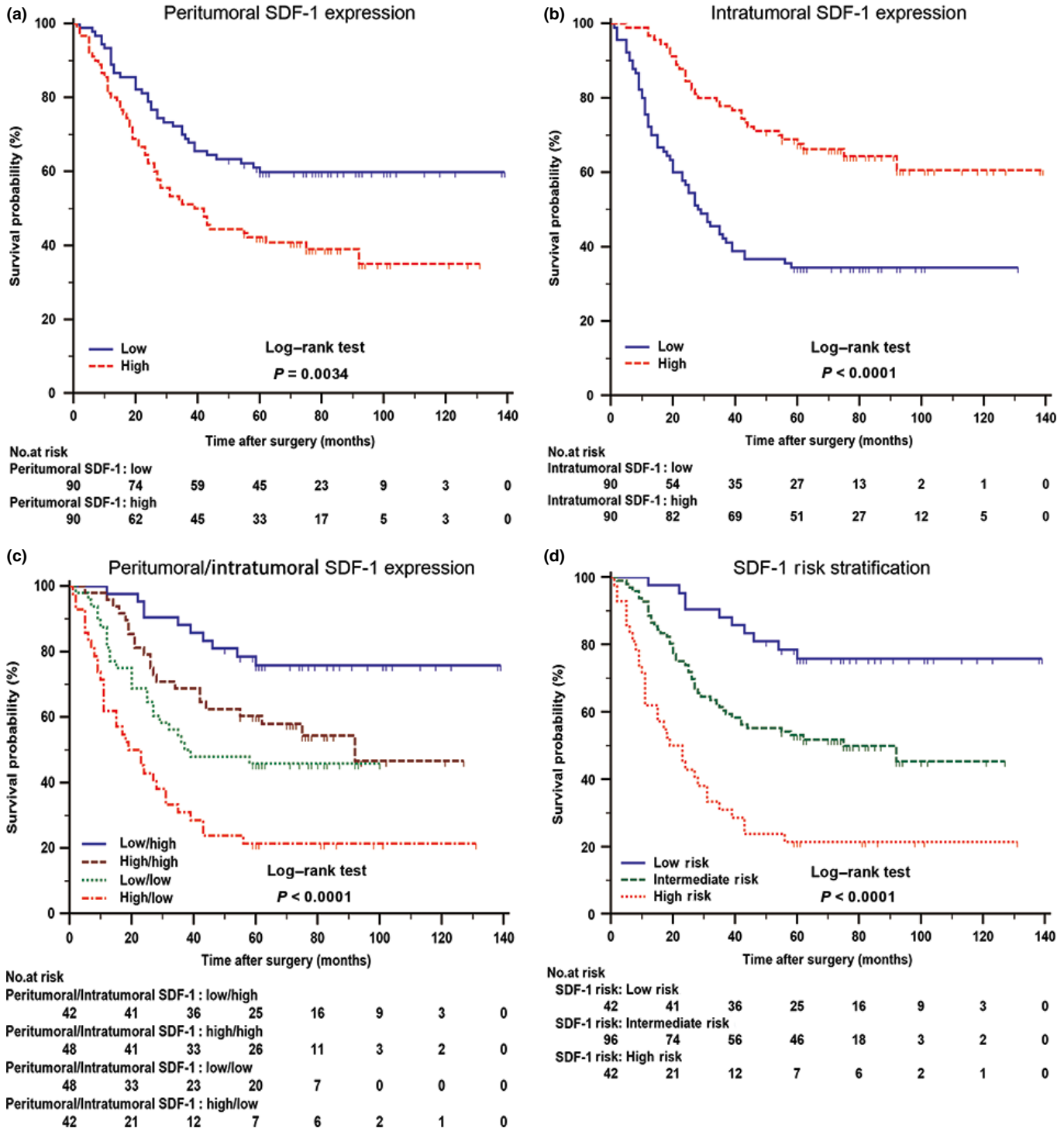

Fig. 2. Kaplan–Meier analysis of overall survival according to stromal cell-derived factor 1 (SDF-1) expression in patients with gastric cancer. Kaplan–Meier analysis of overall survival according to peritumoral SDF-1 expression ($P = 0.0034$) (a), intratumoral SDF-1 expression ($P < 0.0001$) (b) and combined peritumoral/intratumoral SDF-1 expression ($P < 0.0001$) (c). Patients were categorized into three risk groups according to their peritumoral/intratumoral SDF-1 expression signature: low risk, peritumoral low/intratumoral high; intermediate risk, peritumoral high/intratumoral high, and peritumoral low/intratumoral low; and high risk, peritumoral high and intratumoral low. Overall survival differed ($P < 0.0001$) among the three SDF-1 risk stratification groups (d) according to Kaplan–Meier analysis. *P*-values were determined by the log–rank test.

ritumoral low/intratumoral low ($n = 96$); and high risk group, peritumoral high/intratumoral low ($n = 42$). The OS among the three risk groups was significantly different ($P < 0.0001$). The SDF-1 risk stratification system has better discriminative power for clinical outcomes, the bootstrapped C-index was 0.668 compared with 0.640 for intratumoral SDF-1 or 0.577 for peritumoral SDF-1.

Univariate and multivariate Cox analysis. In order to identify the prognostic significance of clinicopathological factors for OS, univariate Cox regression analysis was carried out. Tumor

Table 2. Univariate and multivariate cox regression analyses for overall survival in patients with gastric cancer (*n* = 180)

Factors	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age, years†		0.360		
≤63	1 (reference)			
>63	1.212 (0.803 to 1.830)			
Gender		0.570		
Female	1 (reference)			
Male	0.878 (0.559 to 1.377)			
Localization		0.127		
Middle *versus* proximal	0.594 (0.320 to 1.101)	0.098		
Distal *versus* proximal	0.957 (0.558 to 1.642)	0.875		
Differentiation		0.308		
Moderate *versus* well	3.382 (0.463 to 24.731)	0.230		
Poor *versus* well	4.015 (0.555 to 29.035)	0.169		
Lauren classification		0.208		
Intestinal	1 (reference)			
Diffuse	1.314 (0.859 to 2.010)			
Tumor size, cm†		**0.002**		0.322
<4	1 (reference)		1 (reference)	
≥4	1.969 (1.290–3.006)		1.246 (0.806–1.928)	
T classification		**<0.001**		**0.006**
T2 *versus* T1	1.073 (0.097–11.839)	0.954	0.628 (0.055–7.159)	0.708
T3 *versus* T1	8.964 (1.736–46.274)	**0.009**	8.219 (1.555–43.429)	**0.013**
T4 *versus* T1	18.170 (4.459–74.042)	**<0.001**	7.037 (1.589–31.161)	**0.010**
N classification		**<0.001**		**0.004**
N1 *versus* N0	3.071 (1.437–6.566)	**0.004**	1.460 (0.647–3.297)	0.362
N2 *versus* N0	6.289 (2.945–13.429)	**<0.001**	3.413 (1.488–7.831)	**0.004**
N3 *versus* N0	7.982 (3.966–16.066)	**<0.001**	2.933 (1.320–6.513)	**0.008**
Distant metastasis		**<0.001**		**0.009**
No	1 (reference)		1 (reference)	
Yes	5.746 (2.424–13.617)		3.333 (1.348–8.243)	
SDF-1 risk		**<0.001**		**<0.001**
Intermediate *versus* low	2.634 (1.332–5.209)	**0.036**	2.350 (1.169–4.723)	**0.016**
High *versus* low	6.416 (3.150–13.067)	**<0.001**	5.004 (2.395–10.453)	**<0.001**

CI, confidence interval; N, lymph node; T, tumor depth. †Split at median. Patients were categorized into three risk groups according to peritumoral/intratumoral stromal cell-derived factor 1 (SDF-1) expression signature: low, peritumoral low/intratumoral high; intermediate, peritumoral high/intratumoral high, and peritumoral low/intratumoral low; and high, peritumoral high and intratumoral low. Bold values indicate significance.

size ($P < 0.002$), T classification ($P < 0.001$), N classification ($P < 0.001$), distant metastasis ($P < 0.001$), and SDF-1 risk ($P < 0.001$) were defined as risk factors that may affect the OS of gastric cancer patients (Table 1). After adjustment of covariate factors by using multivariate Cox analysis, we identified T classification ($P = 0.006$), N classification ($P = 0.004$), distant metastasis ($P = 0.009$), and SDF-1 risk ($P < 0.001$) as independent prognostic factors for OS (Table 2).

Predictive nomogram for OS of patients with gastric cancer. To provide a quantitative model to evaluate patient risk for OS, a predictive nomogram was generated by combining all proven prognostic factors after Akaike information criterion selection (Fig. 3a). The nomogram was built by selecting the most powerful independent prognostic factors according to the Akaike information criterion for reduced OS after covariates adjustment. The hazard ratio for each factor was turned into points according to their contribution to reduced OS adjusted by tumor invasion depth. For example, tumor invasion depth has a significant correlation with lymph node metastasis ($P < 0.0001$). The deeper the tumor invaded into the gastric wall, the more frequent lymph node metastasis was found. Thus, the hazard ratio of lymph node metastasis for reduced OS may be adjusted by tumor invasion depth, making the corresponding scores relatively lower in the nomogram. The theory was applied to all the selected prognostic factors. In the nomogram, a higher total score indicates worse survival probability. The calibration curve for predicted 5-year OS performed well with the ideal model (Fig. 3b). The bootstrapped C-index for the prognostic accuracy of the nomogram was 0.780 compared with 0.687 for TNM staging system. The time-dependent ROC curve showed higher sensitivity and specificity for predicting OS (Fig. 3c). All these results showed that the nomogram has better performance for predicting OS.

Discussion

Numerous studies have suggested that many epithelial tumor cells may exploit the chemokine systems that normally regulate leukocyte trafficking to metastasize to distant organs.[25–27] However, the prognostic values of chemokine expression in malignant tumors, especially in gastric cancer,

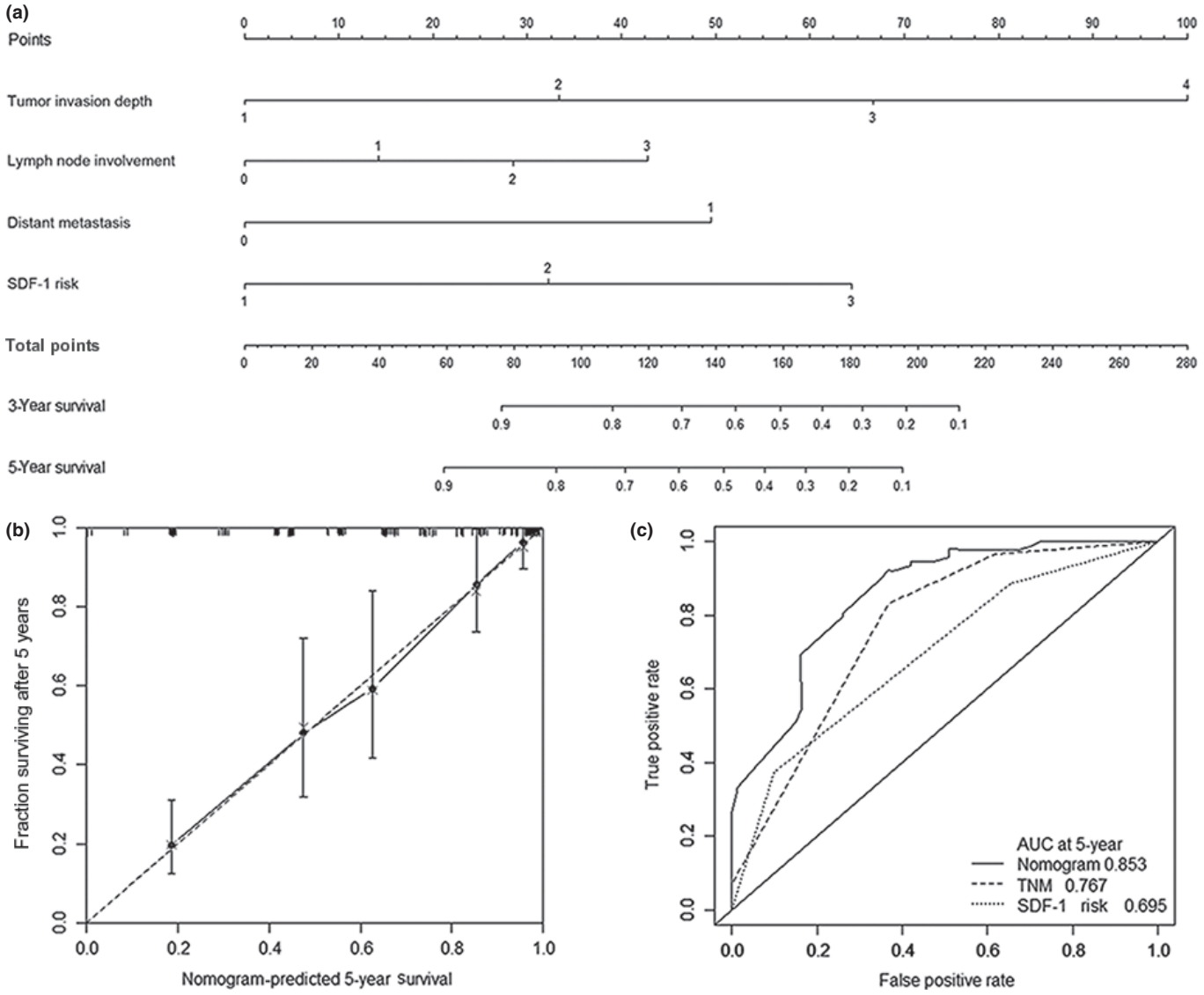

Fig. 3. Prognostic nomogram generated for predicting overall survival in patients with gastric cancer. (a) Predictive nomogram for overall survival was generated by combining proven independent prognostic factors including tumor invasion depth (1 = T1, 2 = T2, 3 = T3, 4 = T4), lymph node involvement (0 = N0, 1 = N1, 2 = N2, 3 = N3), distant metastasis (0 = M0, 1 = M1), and stromal cell-derived factor 1 (SDF-1) risk (1, low risk; 2, intermediate risk; 3, high risk). (b) Calibration plot for nomogram predicted 5-year survival and observed survival. The nomogram performed well with the ideal model. (c) Time-dependent receiver operating characteristic curves by nomogram, TNM stage, and SDF-1 risk for 5-year overall survival probability.

have not been well-defined. In the present study, we have demonstrated the prognostic power of SDF-1 expression in patients with gastric cancer, and categorized patients into three risk groups according to peritumoral/intratumoral SDF-1 expression signature. The SDF-1 risk stratification system was proved to be an independent prognostic factor that can be incorporated with TNM staging variables to generate a predictive nomogram for OS. The established nomogram showed better performance in predicting clinical outcomes for patients with gastric cancer after surgical resection. However, these results need a larger, multicentered dataset to be validated.

Previous studies into the relationship between intratumoral SDF-1 expression and clinical features in gastric cancer have generated diametrically opposite results. Zhi *et al.* found that decreased SDF-1 expression in gastric cancer was significantly associated with aggressive lymph node metastasis and histological grade,[20] whereas Ishigami *et al.*[28] showed relatively higher SDF-1 expression in gastric cancer tissues correlated with aggravated lymph node metastasis, tumor invasion, lymphatic invasion, tumor diameter, and clinical stage. These differences may arise from the antibody and method used in defining SDF-1 positive staining. Here, in this study, we used a specific antibody for SDF-1 staining confirmed by peptide competition, and we used quantitative methods to define staining intensity to minimize the information loss derived from semiquantitative methods. In the present study, we found that SDF-1 expression was significantly downregulated in tumor tissues compared with peritumor tissues. Low intratumoral SDF-1 expression and high peritumoral SDF-1 expression were both correlated with tumor invasion and lymph node metastasis; low intratumoral SDF-1 expression also correlated with tumor size, tumor

differentiation, and clinical stage. The SDF-1 risk stratification system derived from peritumoral/intratumoral SDF-1 expression signatures applied well in discriminating patients with different prognoses compared with intratumoral SDF-1 or peritumoral SDF-1 alone. It appears that SDF-1 is secreted by peritumor tissues and flows through lymphatic or venous routes to gastric tumor cells. This paracrine mechanism would result in favorable conditions for CXCR4-expressed gastric cancer cells to metastasize to the SDF-1 gradient. These results suggested that gastric cancer cells with low SDF-1 expression may have a selective advantage to receive paracrine SDF-1 signals, promoting their growth, and driving more active metastasis to ectopic sources of the CXCR4 ligand, therefore, participating in regulating tumor cell biological phenotypes.

Although our study found that intratumoral SDF-1 expression correlated with patient outcomes, the exact mechanisms underlying this phenomenon are still unknown. Previous studies pointed out that the endogenous SDF-1 derived from epithelial cells was in marked contrast to exogenous ligand, which inhibits tumor metastasis through increased anoikis.[29] Loss of SDF-1 with maintained expression of CXCR4 confers tumor cells a phenotype similar to that of circulating highly migratory leukocytes and lymphocytes, facilitating the receipt of the paracrine SDF-1 signal. Aberrant methylation of the CpG island of the *SDF-1* gene may be a possible mechanism for the downregulation, and treatment with demethylating agent 5-aza-2′-deoxycytidine partly restored SDF-1 expression in gastric cancer cell lines, and suppressed cell invasion.[20] Consistent with previous studies, our study showed that the endogenous SDF-1 expressed in tumor tissues was downregulated compared with peritumor tissues, and correlated with tumor progression. These results may shed light on the establishment of a metastasis model of gastric cancer.

The TNM staging system has been used for decades to predict clinical outcomes for patients with gastric cancer. However, controversies exist about whether additional risk factors, other than the TNM factors, are important parameters to predicting clinical outcomes. In this study, we have proved the prognostic significance of SDF-1 expression. Based on these results, a predictive model that integrated SDF-1 risk and TNM staging variables was constructed. In the constructed nomogram, the predictive power for OS was stronger compared with TNM stage or SDF-1 risk alone. These results implied that incorporation of additional risk factors into the well-established TNM staging system may add some prognostic information to better predict clinical outcomes. However, we did not evaluate the SDF-1 receptor, CXCR4, in the present study. Numerous studies, including our previous study, have shown that CXCR4 overexpression was negatively correlated with clinical outcomes of gastric cancer patients.[17,18,30,31] Thus, combined analysis of the SDF-1/CXCR4 axis may add more prognostic information to the current TNM staging model. In addition, detailed information about recurrence was not available, which is a defect of this study, making the investigation of the relation between SDF-1 expression and recurrence unachievable. Further investigation about the relation between SDF-1 and recurrence and underlying molecular mechanisms will be investigated in our ongoing study.

Along with the prognostic significance for predicting clinical outcomes, targeting chemokine-mediated tumor cell microenvironment interaction has been proved to play a crucial role in sensitizing malignant tumors to chemotherapy.[32–34] Gastric cancer may use the paracrine SDF-1/CXCR4 axis to confer tumor cells the ability to survive and resist apoptosis induced by cytotoxic drugs. Therefore, targeting the SDF-1/CXCR4 axis by specific inhibitors, such as AMD3100, may sensitize gastric cancer to chemotherapy.

In conclusion, our present study has proved the prognostic values of peritumoral and intratumoral SDF-1 expression, identifying SDF-1 risk as an important prognostic factor for OS. Incorporation of SDF-1 risk into the current TNM staging system could refine the risk stratification system for predicting OS in patients with gastric cancer, and targeting the SDF-1/CXCR4 axis may open a new avenue for treatment of gastric cancer in combination with traditional cytotoxic drugs, especially in patients with higher metastasis potential.

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Relationship between mRNA and protein expression levels of stromal cell-derived factor 1 (SDF-1) in patients with gastric cancer (n = 40).

Fig. S2. Association between stromal cell-derived factor 1 (SDF-1) expression and overall survival of patients with gastric cancer according to tumor invasion depth.