Human Pathology (2019) **84**, 44–51

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Overexpression of ADAMTS-2 in tumor cells and stroma is predictive of poor clinical prognosis in gastric cancer☆,☆☆

Chunyi Jiang MD[a,b,1], Yi Zhou MSc[c,1], Yiyang Huang MSc[d], Yan Wang MSc[b], Wei Wang MSc[b], Xiaoling Kuai MD[e,*]

[a]*Department of Clinical Bio-bank, Nantong University Affiliated Hospital, Nantong, Jiangsu 226001, China*
[b]*Department of Pathology, Nantong University Affiliated Hospital, Nantong, Jiangsu 226001, China*
[c]*School of Medicine, Nantong University, Nantong, Jiangsu 226001, China*
[d]*Nantong Middle School, Jiangsu 226001, China*
[e]*Department of Gastroenterology, Nantong University Affiliated Hospital, Nantong, Jiangsu 226001, China*

Received 3 May 2018; revised 25 August 2018; accepted 29 August 2018

Keywords:
ADAMTS-2;
Immunohistochemistry;
Tissue microarray;
Gastric cancer;
Prognosis

Summary ADAMTS-2 is a member of the ADAMTS family and is a procollagen *N*-proteinase. The objective of our research is to explore the prognostic significance of ADAMTS-2 in gastric carcinoma. A total of 655 samples with full clinicopathological data were investigated in this study. Tissue microarray immunohistochemistry analysis was used to analyze the relationship between clinicopathological characteristics and ADAMTS-2 expression. Oncomine and Kaplan-Meier plotters were performed for the relationship analysis between prognosis and ADAMTS-2 expression in patients with gastric cancer. Compared with that of normal tissues, the ADAMTS-2 protein expression was remarkably higher in gastric cancer cells and fibroblast cells. The results of univariate analysis indicated that the expression of ADAMTS-2 in tumor cells and fibroblast cells, Laurén classification, TNM grade, and carcinoembryonic antigen level in gastric cancer were all correlated with overall survival. The results of multivariate analysis indicated that the high expression of ADAMTS-2 in gastric cancer cells and fibroblast cells both were independent prognostic factors. Therefore, ADAMTS-2 may be a potential biomarker for assessing the prognosis of gastric carcinoma.

☆ Competing interests: All authors state that they have no conflict of interest.

☆☆ Funding/Support: This work was supported by Key Medical Talents (ZDRCA2016051) and Six High Level Talents Project (LGY2016038) of Jiangsu Health Planning Commission, Jiangsu, China, and Science and Technology Projects (MS32017001, MS12016031) of Nantong, Jiangsu, China.

* Corresponding author at: Affiliated Hospital of Nantong University, No. 20, XISI Rd, Nantong 226001, Jiangsu, China.
E-mail addresses: jiangchunyi1219@126.com (C. Jiang), 80548088@qq.com (Y. Zhou), 2531312483@qq.com (Y. Huang), yisheng_wangyan@126.com (Y. Wang), wangjiawei666@126.com (W. Wang), kuaixiaoling1@163.com (X. Kuai).

[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.humpath.2018.08.030
0046-8177/

1. Introduction

Gastric cancer is the fourth most common malignant tumor type worldwide and the second most common cancer-related cause of death. The average 5-year survival rate of gastric carcinoma is less than 20% [1]. More than 50% of gastric carcinoma cases occur in East Asia [2,3]. Although the incidence and mortality rates of gastric cancer have declined over the last century, gastric cancer is still a focus of current research. The median survival time of patients with advanced gastric cancer is only around 1 year [4]. With the advancement of molecular biology approaches, the molecular characteristics of the gastric carcinoma can be better understood. Highly specific and sensitive biomarkers are essential for improving disease prevention, diagnosis, targeting therapy, and prognosis prediction [5].

ADAMTS (a disintegrin and metalloproteinase with thrombospondin type I domain) proteins are zinc metalloendopeptidases and involved in cancer and tumor protection [6]. The ADAMTS protein family includes 19 members that are grouped according to their known substrate [6]. ADAMTS-2 is one procollagen *N*-proteinase, and ADAMTS-2 gene is located on human chromosome 5q23-24, encoding 1211 amino acids [7]. Overexpression, mutation, or epigenetic silencing of different ADAMTS genes has been identified in tumors of different origins, suggesting that these metalloproteases have a direct effect on the development of cancer.

The epithelial-fibroblast interactions are significant for the development of cancer [8,9]. The overexpression of ADAMTS-2 protein in tumor fibroblast cells has not been well studied to date. In our study, we found that ADAMTS-2 was overexpressed in the fibroblast cells of gastric cancer.

Expression of ADAMTS-2 in gastric carcinoma cells and fibroblast cells and its correlation with patients' clinical and pathological characteristics were investigated with tissue microarray immunohistochemistry (TMA-IHC) analysis. Then, the clinical and pathological features of ADAMTS-2 and the prognostic significance to gastric cancer patients were further studied.

2. Materials and methods

2.1. Gastric cancer patient samples and clinicopathological data

The specimens of gastric cancer were acquired through Clinical Biobank in Affiliated Hospital of Nantong University. Tumor tissues collected from patients who underwent surgery from June 2004 to July 2009 were fixed by using formalin and were embedded within paraffin. This study included 655 samples. All sections were examined by at least 2 independent pathologists. Before the surgery, all the patients in this study had not received chemotherapy, immunotherapy, or radiation therapy. Overall survival was classified as the period from the first biopsy date to the death date. Tumors were staged based on the World Health Organization (WHO) standards [10]. The protocol of research gained approval from the Human Ethics Committee in Affiliated Hospital of Nantong University.

2.2. Construction of TMA and IHC analysis

We used TMA-IHC to measure the expression of ADAMTS-2 protein in tissue blocks. Core tissue biopsies (diameter of 2 mm) taken from single formalin-fixed, paraffin-embedded slices were rearrayed in recipient paraffin masses by using one Tissue Microarray System (Quick-Ray, UT06; UNITMA, Seoul, Korea). The slides of TMA were incubated along with anti–ADAMTS-2 antibody (1:100, Atlas, HPA02844) at 4°C overnight, and then antirabbit biotinylated antibody was applied to be a secondary antibody at indoor temperature for 2 hours. Phosphate-buffered saline was used to be a negative control.

Staining intensity index and positive rate of ADAMTS-2–positive cells for each section were scored by at least 2 blinded pathologists. Staining intensity scores were 0 (−, no staining), 1 (+, weak staining), 2 (++, moderate staining), and 3 (+++, intense staining). ADAMTS-2–positive rate score ranged between 0 and 100. The product of positive rate score and intensity score index, which is regarded as the final IHC score, was calculated from 0 to 300. X-tile software (The Rimm Lab in Yale University, New Haven, CT; http://www.tissuearray.org/rimmlab) [11] was applied to set cutoff point for expression score of ADAMTS-2, which has statistical significance in overall survival. ADAMTS-2 expression levels were graded by using a 2-grade scoring system; the expression scores were as follows: low expression, 0 to 159; high expression, 160 to 300 for ADAMTS-2 in tumor cells; low expression, 0 to 179; and high expression, 180 to 300 for ADAMTS-2 in fibroblast cells.

2.3. Bioinformatic analysis

Oncomine (https://www.oncomine.org), an RNA and DNA sequence database, was used for assessing expression level of ADAMTS-2 gene within the gastric cancer samples. At the time of analysis, Oncomine had incorporated 65 sets of gene expression data sets consisting of almost 48 million gene expression data points, constituting more than 4700 microarray experiments. The differential expression analysis of the most prevalent cancer types and their respective normal tissues can be explored, as well as various cancer hypotypes and clinical and pathology analysis. The ADAMTS-2 messenger RNA (mRNA) expression of gastric cancer samples was compared with that of other gastric samples using the method.

Relevance between the expression of ADAMTS-2 mRNA and the overall survival of patients with gastric carcinoma was furthered explored by means of an online survival function analysis tool called Kaplan-Meier plotter (KM plotter; http://kmplot.com/analysis/). The KM plotter includes transcriptome information of 1065 gastric cancer samples (54 675 genes) along with 33-month average follow-up time [12].

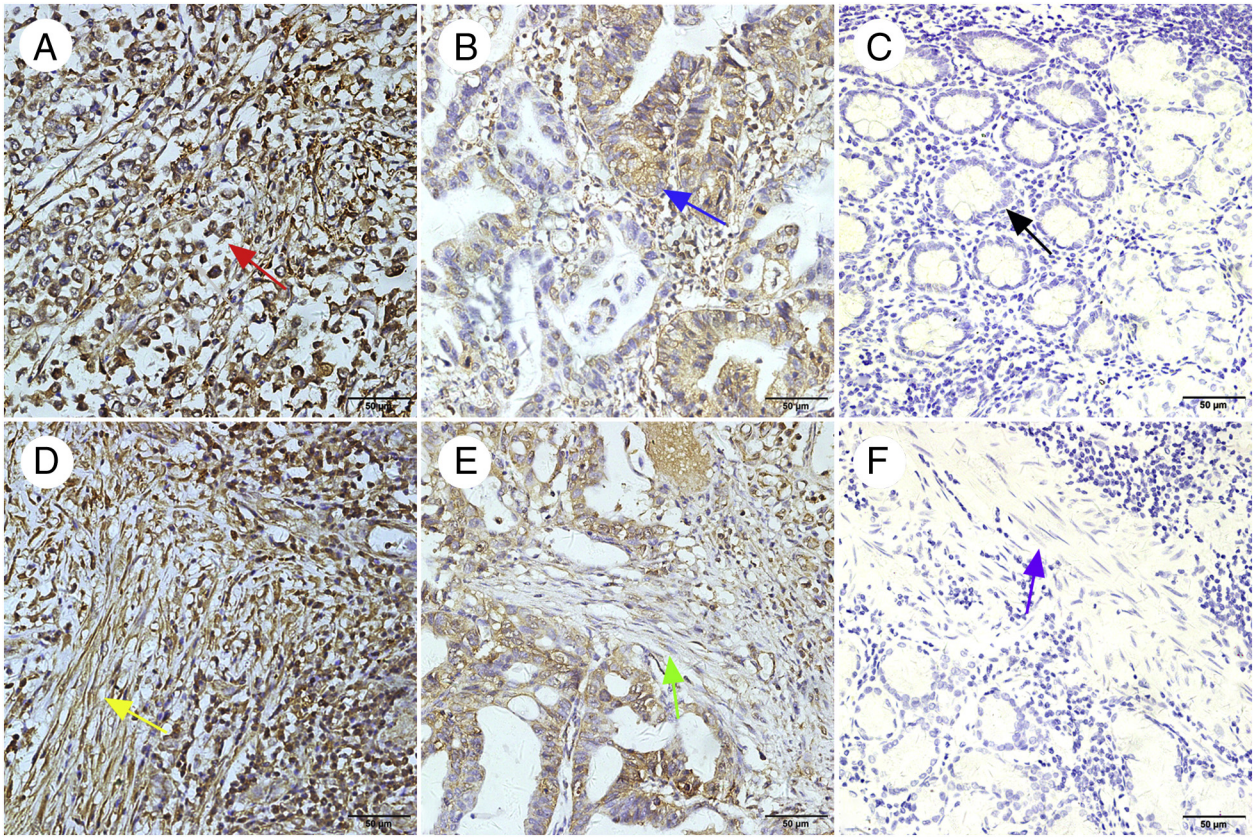

Fig. 1 Representative image of the expression ADAMTS-2 in benign and malignant gastric tissues through IHC. A, Strong positive immunohistochemical staining of ADAMTS-2 in tumor cell cytoplasm (red arrow) in diffuse gastric cancer samples. B, Weak positive immunohistochemical staining of ADAMTS-2 in tumor cell cytoplasm (blue arrow) in intestinal-type gastric cancer samples. C, ADAMTS-2–negative immunohistochemical staining of epithelial cells (black arrow) in chronic gastritis samples. D, Strong positive immunohistochemical staining of ADAMTS-2 in fibroblasts (yellow arrow) in diffuse gastric cancer samples. E, Weak positive immunohistochemical staining of ADAMTS-2 in fibroblasts (green arrow) in intestinal-type gastric cancer samples. F, ADAMTS-2–negative immunohistochemical staining of fibroblasts (purple arrow) in chronic gastritis samples. Original magnification ×400 (bar, 50 μm).

2.4. Statistical analysis

The relationship between ADAMTS-2 expression and clinicopathological data was evaluated using χ^2 tests. Prognosis-related factors in univariate model were assessed using a multivariate Cox regression model. The survival curves were estimated using the Kaplan-Meier analysis. For each analysis, a P value of less than .05 was considered remarkable in statistics. SPSS 20 (SPSS, Chicago, IL) software package and STATA 12.0 (Stata Corporation, College Station, TX) were adopted to perform the statistical analyses.

Table 1 Expression of ADAMTS2 in gastric benign and malignant tissues

Characteristic	n (n = 655)	Cytoplasmic staining of ADAMTS2 in tumor cells			
		Low or no expression	High expression	Pearson χ^2	P
Chronic gastritis	20	15 (75.00)	5 (25.00)	81.618	<.001 *
Intestinal metaplasia	41	33 (80.49)	8 (19.51)		
Low-grade intraepithelial neoplasia	29	23 (79.31)	6 (20.69)		
High-grade intraepithelial neoplasia	24	14 (58.33)	10 (41.67)		
Cancer	485	187 (38.56)	298 (61.44)		
Surgical margin (gastric cancer patients)	56	50 (89.29)	6 (10.71)		

* $P < .05$.

Table 2 Relationship between ADAMTS-2 expression and clinical and pathological data of gastric cancer patients

Characteristic	n	Cytoplasmic staining of ADAMTS2 in tumor cells				Fibroblast staining of ADAMTS2			
		Low or no expression	High expression	Pearson χ^2	*P*	Low or no expression	High expression	Pearson χ^2	*P*
Total	485	187 (38.56)	298 (61.44)			236 (48.66)	249 (51.34)		
Sex				3.067	.080			0.483	.487
Male	361	131 (36.29)	230 (63.71)			179 (49.58)	182 (50.42)		
Female	124	56 (45.16)	68 (54.84)			57 (45.97)	67 (54.03)		
Age (y)				6.301	.012*			1.233	.267
<60	179	82 (45.81)	97 (54.19)			93 (51.96)	86 (48.04)		
≥60	306	105 (34.31)	201 (65.69)			143 (46.73)	163 (53.27)		
Laurén classification				39.325	<.001*			27.474	<.001*
Intestinal type (well differentiated and moderately differentiated)	193	103 (53.37)	90 (46.63)			120 (62.18)	73 (37.82)		
Diffuse type (poorly differentiated)	160	41 (25.62)	119 (74.38)			67 (41.88)	93 (58.13)		
Mixed type (moderately and poorly differentiated)	121	35 (28.93)	86 (71.07)			42 (34.71)	79 (65.29)		
Uncertain type[a]	11	8	3			7	4		
Vascular invasion				6.293	.012*			1.839	.175
No	350	147 (42.00)	203 (58.00)			177 (50.57)	173 (49.43)		
Yes	135	40 (29.63)	95 (70.37)			59 (43.70)	76 (56.30)		
T				10.603	.005*			12.937	.002*
Tis and T1	64	33 (51.56)	31 (48.44)			38 (59.38)	26 (40.63)		
T2	99	46 (46.46)	53 (53.54)			60 (60.61)	39 (39.39)		
T3 and T4	322	108 (33.54)	214 (66.46)			138 (42.9)	184 (57.14)		
N				22.908	<.001*			13.671	.003*
0	185	95 (51.35)	90 (48.65)			107 (57.84)	78 (42.16)		
1	92	34 (36.96)	58 (63.04)			47 (51.09)	45 (48.91)		
2	102	29 (28.43)	73 (71.57)			39 (38.24)	63 (61.76)		
3	106	29 (27.36)	77 (72.64)			43 (40.57)	63 (59.43)		
M				0.448	.503			0.277	.599
M0	449	175 (38.98)	274 (61.02)			220 (49.00)	229 (51.00)		
M1a and M1b	36	12 (33.33)	24 (66.67)			16 (44.44)	20 (55.56)		
TNM stage				28.532	<.001*			19.138	.002*
I (0, Ia, and Ib)	104	58 (55.77)	46 (44.23)			68 (65.38)	36 (34.62)		
IIa	101	47 (46.53)	54 (53.47)			52 (51.49)	49 (48.51)		
IIb	67	25 (37.31)	42 (62.69)			31 (46.27)	36 (53.73)		
IIIa	87	24 (27.59)	63 (72.41)			35 (40.23)	52 (59.77)		
IIIb	81	20 (24.69)	61 (75.31)			34 (41.98)	47 (58.02)		
IIIc and IV	45	13 (28.89)	32 (71.11)			16 (35.56)	29 (64.44)		
CEA				4.118	.128			2.573	.276
No	203	89 (43.84)	114 (56.16)			104 (51.23)	99 (48.77)		
Yes	63	22 (34.92)	41 (65.08)			25 (39.68)	38 (60.32)		
Unknown	219	76	143			107	112		
CA199				4.712	.095			1.844	.398
0	219	96 (43.84)	123 (56.16)			110 (50.23)	109 (49.77)		
1	39	13 (33.33)	26 (66.67)			15 (38.46)	24 (61.54)		
Unknown	227	78	149			111	116		
Her2				16.455	.001*			7.312	.063
0	354	154 (43.50)	200 (56.50)			180 (50.85)	174 (49.15)		
1	33	12 (36.36)	21 (63.64)			19 (57.58)	14 (42.42)		
2	52	13 (25.00)	39 (75.00)			22 (42.31)	30 (57.69)		
3	46	8 (17.39)	38 (82.61)			15 (32.61)	31 (67.39)		

[a] Squamous cell cancer, 4 cases; adenosquamous cancer, 4 cases; neuroendocrine cancer, 3 cases.

* $P < .05$;

Table 3 Univariate and multivariable analyses of prognostic features for 5-year survival rate in gastric cancer

	Univariate analysis				Multivariate analysis			
	HR	$P > \|z\|$	95% CI		HR	$P > \|z\|$	95% CI	
Expression of ADAMTS2 in tumor cells: high *vs* low	2.546	<.001 *	1.920	3.375	1.891	<.001 *	1.401	2.553
Expression of ADAMTS2 in fibroblast cells: high *vs* low	1.975	<.001 *	1.537	2.538	1.377	.019 *	1.055	1.797
Age: ≤60 y *vs* >60 y	1.239	.104	0.957	1.604				
Sex: male *vs* female	1.016	.913	0.767	1.345				
Laurén classification: intestinal type *vs* diffuse type *vs* mixed type *vs* uncertain type	1.420	<.001 *	1.240	1.625	1.299	.001 *	1.120	1.508
Vascular invasion: yes *vs* low	1.284	.063	0.986	1.671				
TNM stage: 0, Ia and Ib *vs* IIa *vs* IIb *vs* IIIa *vs* IIIb *vs* IIIc and IV	1.606	<.001 *	1.484	1.738	1.563	<.001 *	1.442	1.696
T: Tis and T1 *vs* T2 *vs* T3 and T4	2.322	<.001 *	1.832	2.942				
N: 0 *vs* 1 *vs* 1 *vs* 2 *vs* 3	1.704	<.001 *	1.533	1.894				
M: M0 *vs* M1a and M1b	3.404	<.001 *	2.306	5.025				
CEA level: ≤5 *vs* >5	1.144	.041 *	1.005	1.301	1.119	.092	0.982	1.276
CA199 level: ≤37 *vs* >37	1.097	.147	0.968	1.243				
Her2: 0 *vs* 1 *vs* 2 *vs* 3	1.040	.505	0.926	1.169				

* $P < .05$.

3. Results

3.1. ADAMTS-2 protein localization and expression

TMA-IHC analysis was performed to detect the expression of ADAMTS-2 in 655 on-filed gastric tissue blocks. Fig. 1 demonstrates the representative immunohistochemical ADAMTS-2 staining. ADAMTS-2–positive staining was mostly in the cytoplasm of the tumor and fibroblast cells. Tumor heterogeneity is widely considered to be one of the most basic features of malignant tumors. Because of the heterogeneity of gastric cancer, the cell positive rate is not consistently all positive or negative in the same tissue point, but a different percentage of positive rat (Fig. 1B). ADAMTS-2 expression in diffuse gastric cancer is stronger than that in the intestinal type (Fig. 1). All gastric specimens were scored and classified on the basis of the cutoff point of ADAMTS-2 expression derived from the X-tile software program.

Compared with those of chronic gastritis tissue (5/20; 25.0%), intestinal metaplasia tissue (8/41; 19.51%), low-grade intraepithelial neoplasia tissue (6/29; 20.69%), and high-grade intraepithelial neoplasia tissue (10/24; 41.67%) samples, ADAMTS-2 was up-regulated in the cytoplasm of gastric cancer tumor cells (298/485; 61.44%). Data analyzed using the χ^2 test showed statistical significance ($\chi^2 = 81.618$, $P < .001$; Table 1).

3.2. Relationship between the expression of ADAMTS-2 and clinical and pathological characteristics of gastric cancer patients

The relationship between the protein expression of ADAMTS-2 and the clinical and pathological data of gastric cancer is shown in Table 2. It was demonstrated that the up-regulated cytoplasmic expression of ADAMTS-2 in tumor cells was significantly correlated with age ($P = .012$), Laurén

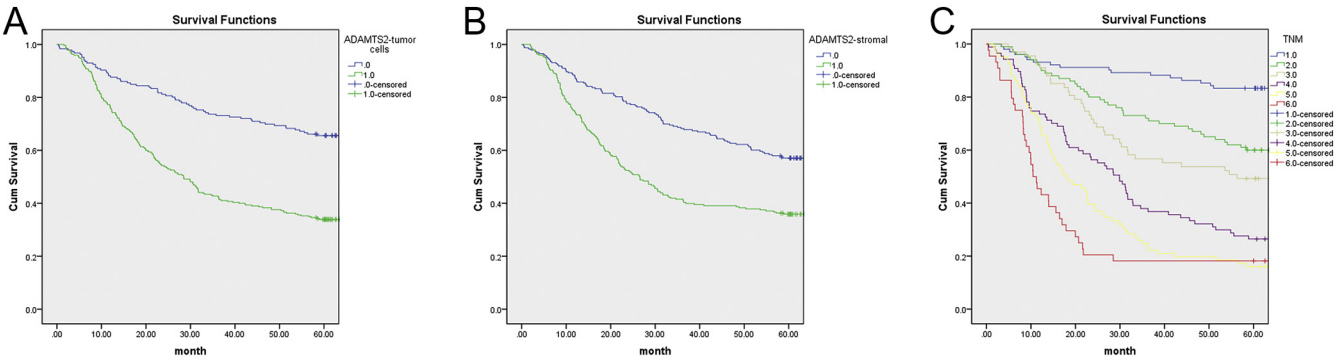

Fig. 2 Survival curves of gastric cancer patients according to ADAMTS-2 expression by Kaplan-Meier analysis. A, Patients with high expression of ADAMTS-2 in tumor cells (green line, 1) have a lower overall survival rate compared with those with low or no expression of ADAMTS-2 in tumor cells (blue line, 0). B, Patients with high fibroblast expression of ADAMTS-2 (green line, 1) exhibit a lower overall survival rate compared with those with low or no fibroblast expression of ADAMTS-2 (blue line, 0). C, Patients with advanced TNM grade have a remarkably lower overall survival rate compared with those with early TNM grade.

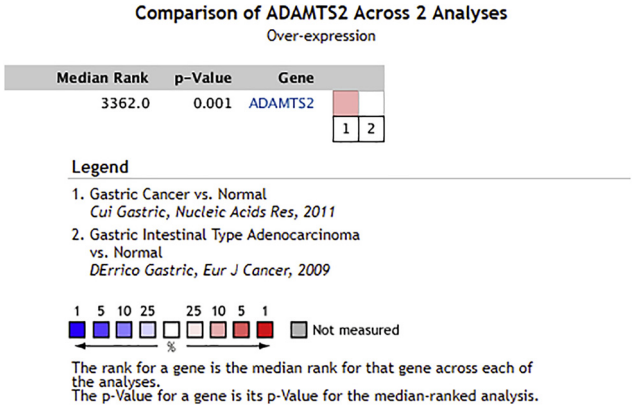

Fig. 3 Oncomine database analysis of ADAMTS-2 expression in gastric cancer patients. Two analyses were performed to examine the mRNA level of ADAMTS-2. The color intensity shows the corresponding levels of ADAMTS-2. The red column indicates up-regulation of ADAMTS-2 mRNA.

classification ($P < .001$), vascular invasion ($P = .012$), tumor status (T; $P = .005$), lymph node status (N; $P < .001$), TNM grade ($P < .001$), and Her2 ($P = .001$; Table 2). However, high ADAMTS-2 expression in fibroblast tissue had a significant association with Laurén classification ($P < .001$), tumor status (T; $P = .002$), lymph node status (N; $P = .003$), and TNM grade ($P = .002$; Table 2).

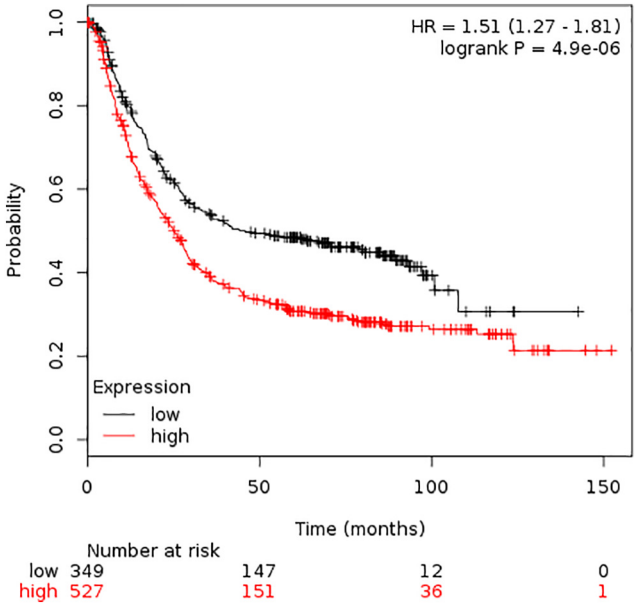

Fig. 4 Significance of ADAMTS-2 expression in prognosis of gastric carcinoma patients. The Kaplan-Meier plotter shows significantly lower survival rates in patients with high ADAMTS-2 tumor levels than in patients with low levels of ADAMTS-2 (n = 1065; log-rank test, $P < .001$).

3.3. Association between patient survival, ADAMTS-2 protein expression, and clinicopathological characteristics

Relative factors of gastric cancer prognosis were examined using multivariate and univariate analyses. In univariate analysis, some factors demonstrated meaningful correlation with overall survival, which included the expression of ADAMTS-2 in tumor cells and fibroblast cells, Laurén classification, tumor status, lymph node status, distant metastasis, TNM grade, and blood serum carcinoembryonic antigen (CEA) levels (Table 3). In multivariate analysis, up-regulated ADAMTS-2 expression in both tumor cells and fibroblast cells, Laurén classification, and advanced TNM grade were independent prognostic factors for poor prognosis (Table 3). Kaplan-Meier survival analysis indicated that the prognosis of gastric carcinoma patients with high cytoplasmic and fibroblast ADAMTS-2 expression and advanced TNM stage was significantly worse (Fig. 2).

3.4. Up-regulated ADAMTS-2 expression indicates poor prognosis in gastric carcinoma

We also analyzed Oncomine data to evaluate the relationship between survival and ADAMTS-2 expression of the patients with gastric carcinoma. Consistent with our data, the mRNA levels of ADAMTS-2 were overexpressed across 2 data sets (Fig. 3). Moreover, the KM plotter showed that for the overall survival, low expression of ADAMTS-2 was a good prognostic factor (log-rank test, $P < .001$; Fig. 4).

4. Discussion

The ADAMTS are metallopeptidases and disintegrins with a thrombospondin motif that contains a metalloprotease catalytic domain and a carboxy-terminal auxiliary domain [6]. Studies demonstrated that ADAMTS proteases are important to development, procreation, homeostasis, and disease and are related to tumorigenesis and metastasis [13]. The ADAMTS family consists of 19 members; ADAMTS-2 is the most investigated and characterized procollagen *N*-endopeptidase [14,15]. It consists of 4 TSR1 domains and 1 RGD sequence rich in Cys domains that may bind to integrins [16]. The gene mutations of ADAMTS-2 can cause Ehlers-Danlos syndrome dermatosparactic type or type VIIC [17]. ADAMTS-2 deficiency in mice has been shown to be associated with male infertility [18], and the increased expression of ADAMTS-2 has also been demonstrated to be correlated with acute myocardial infarction [19], but potential substrates related to these activities are still unknown. Different ADAMTSs may exert their antitumor effects by blocking Erk phosphorylation, which is usually increased in tumor cells [20]. ADAMTS-2 has been shown to reduce Erk phosphorylation levels in HUVEC cells [21]. Furthermore, it has been reported that ADAMTS-2 can be mediated by transforming growth

factor β1, which has a certain influence on the mechanism of action of growth factors that affect the formation of extracellular matrix. ADAMTS-2 was up-regulated in osteosarcoma cells after transforming growth factor-β treatment [22].

The overall survival rate of advanced gastric cancer is about 1 year, which is a frustrating prognosis, in part because of the high level of biological heterogeneity found in gastric cancer. It is worth noting that intrinsic variation exists not only between patients with the same type of malignancy (intertumor heterogeneity) but also in any individual tumor (intratumoral heterogeneity) [23].Clinical and histologic tumor heterogeneity is reflected by immunohistochemical expression and histopathologic classification of gastric cancer. Because of the heterogeneity of gastric cancer, we took core tissue biopsies of 2 mm in diameter to rule out the effect of heterogeneity on the results. In the same tumor tissue point, the cells showed different percentages of positive. Because tumor plasticity and intercellular and intracellular heterogeneity have been reported as possible factors for treatment failure, it is critical to study gastric cancer heterogeneity to develop more effective therapeutic regimens that can target heterogeneous tumor cell populations.

The morphologic heterogeneity of gastric cancer is very different. A large number of histopathologic classifications proposed over the years have demonstrated the diversity of gastric cancer morphology. Although dating back to 1965, the Laurén classification is still widely accepted and used [24]. It is based on dichotomy, which distinguishes between 2 main types: intestinal gastric cancer, tubular or papillary structures, and diffuse gastric cancer, characterized by poorly viscous and invasive tumor cells, which may or may not have signet-ring cell form. Gastric cancer groups that are not suitable for these 2 main types include mixed and uncertain subtypes. Studies have shown that diffuse gastric cancer seems to be more aggressive than intestinal cancer and has been identified as an independent poor prognostic factor [25,26]. Our study shows that ADAMTS-2 is expressed in diffuse type higher than intestinal type. Up-regulated expression of ADAMTS-2 was significantly correlated with Laurén classification, and Laurén classification was correlated with overall survival.

In this research, we proved that ADAMTS-2 expression in tumor cells was an independent predictor of prognosis in gastric cancer. Moreover, our study indicated that ADAMTS-2 was overexpressed not merely in the cytoplasm of tumor cells but also in the fibroblast cells of gastric carcinoma. Since it has been reported that the genetically stable fibroblast cell types is significant for the microenvironment of tumor, including tumor recurrence and drug resistance [27], increasing numbers of researchers have paid special attention to tumor marker's expression within tumor fibroblast cells and have discovered that they have a close correlation with tumor progression and prognosis [28,29]. It has been reported that some ADAMTSs can also be expressed in stromal cells [30]. Our results show that ADAMTS-2 expression level was up-regulated significantly within fibroblast cells of gastric cancer tissue compared with the corresponding noncancerous tissues. All of the above findings indicate that ADAMTS-2 may be important for gastric cancer development through its expression in both cancer and fibroblast cells.

Nevertheless, there exist limitations in our research. First of all, because this article is a retrospective analysis, specimens' size and quality are low. Second, the underlying mechanisms of ADAMTS-2 affecting the development and prognosis of gastric carcinoma are unclear. Subsequent research should be conducted to discover the biological mechanism regarding ADAMTS-2 role in gastric cancer development. Third, IHC may not accurately measure the expression of ADAMTS-2 within the gastric cancer cells.

In conclusion, our research indicates that, in comparison with normal tissues, ADAMTS-2 expression in both gastric cancer tumor and fibroblast cells was remarkably increased and that up-regulated ADAMTS-2 expression was relevant to poor prognosis. To our knowledge, this is the first time that ADAMTS-2 has been reported as a prognostic factor for the overall survival among gastric carcinoma patients. It is likely that ADAMTS-2 is a brand-new prognostic marker of gastric carcinoma; nevertheless, action mechanism of ADAMTS-2 requires further exploration.

References

[1] Correa P. Gastric cancer: overview. Gastroenterol Clin North Am 2013; 42:211-7.

[2] Yang L. Incidence and mortality of gastric cancer in China. World J Gastroenterol 2006;12:17-20.

[3] Fock KM, Ang TL. Epidemiology of *Helicobacter pylori* infection and gastric cancer in Asia. J Gastroenterol Hepatol 2010;25:479-86.

[4] Koizumi W, Narahara H, Hara T, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 2008;9:215-21.

[5] Zhang H, Qiu J, Ye C, et al. ROR1 expression correlated with poor clinical outcome in human ovarian cancer. Sci Rep 2014;4:5811.

[6] Kelwick R, Desanlis I, Wheeler GN, Edwards DR. The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. Genome Biol 2015;16:113.

[7] Tang BL. ADAMTS: a novel family of extracellular matrix proteases. Int J Biochem Cell Biol 2001;33:33-44.

[8] Angelucci C, Maulucci G, Lama G, et al. Epithelial-stromal interactions in human breast cancer: effects on adhesion, plasma membrane fluidity and migration speed and directness. PLoS One 2012;7:e50804.

[9] Salameh TS, Le TT, Nichols MB, Bauer E, Cheng J, Camarillo IG. An ex vivo co-culture model system to evaluate stromal-epithelial interactions in breast cancer. Int J Cancer 2013;132:288-96.

[10] Xi H, Zhang K, Wei B, Chen L. Significance and contemplation of clinical diagnosis and therapy on the renewal of the eighth edition of gastric cancer TNM staging system. Zhonghua Wei Chang Wai Ke Za Zhi 2017; 20:166-70.

[11] Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res 2004;10:7252-9.

[12] Szasz AM, Lanczky A, Nagy A, et al. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. Oncotarget 2016;7:49322-33.

[13] Wagstaff L, Kelwick R, Decock J, Edwards DR. The roles of ADAMTS metalloproteinases in tumorigenesis and metastasis. Front Biosci 2011; 16:1861-72.

[14] Colige A, Beschin A, Samyn B, et al. Characterization and partial amino acid sequencing of a 107-kDa procollagen I *N*-proteinase purified by

affinity chromatography on immobilized type XIV collagen. J Biol Chem 1995;270:16724-30.
[15] Colige A, Li SW, Sieron AL, Nusgens BV, Prockop DJ, Lapiere CM. cDNA cloning and expression of bovine procollagen I *N*-proteinase: a new member of the superfamily of zinc-metalloproteinases with binding sites for cells and other matrix components. Proc Natl Acad Sci U S A 1997;94:2374-9.
[16] Colige A, Ruggiero F, Vandenberghe I, et al. Domains and maturation processes that regulate the activity of ADAMTS-2, a metalloproteinase cleaving the aminopropeptide of fibrillar procollagens types I-III and V. J Biol Chem 2005;280:34397-408.
[17] Colige A, Nuytinck L, Hausser I, et al. Novel types of mutation responsible for the dermatosparactic type of Ehlers-Danlos syndrome (type VIIC) and common polymorphisms in the ADAMTS2 gene. J Invest Dermatol 2004;123:656-63.
[18] Dubail J, Apte SS. Insights on ADAMTS proteases and ADAMTS-like proteins from mammalian genetics. Matrix Biol 2015;44-46:24-37.
[19] Lee CW, Hwang I, Park CS, et al. Expression of ADAMTS-2, -3, -13, and -14 in culprit coronary lesions in patients with acute myocardial infarction or stable angina. J Thromb Thrombolysis 2012;33:362-70.
[20] Sulzmaier FJ, Ramos JW. RSK isoforms in cancer cell invasion and metastasis. Cancer Res 2013;73:6099-105.
[21] Dubail J, Kesteloot F, Deroanne C, et al. ADAMTS-2 functions as anti-angiogenic and anti-tumoral molecule independently of its catalytic activity. Cell Mol Life Sci 2010;67:4213-32.
[22] Wang WM, Lee S, Steiglitz BM, et al. Transforming growth factor-beta induces secretion of activated ADAMTS-2. A procollagen III *N*-proteinase. J Biol Chem 2003;278:19549-57.
[23] Gullo I, Carneiro F, Oliveira C, Almeida GM. Heterogeneity in gastric cancer: from pure morphology to molecular classifications. Pathobiology 2018;85:50-63.
[24] Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
[25] Qiu MZ, Cai MY, Zhang DS, et al. Clinicopathological characteristics and prognostic analysis of Lauren classification in gastric adenocarcinoma in China. J Transl Med 2013;11:58.
[26] Chen YC, Fang WL, Wang RF, et al. Clinicopathological variation of Lauren classification in gastric cancer. Pathol Oncol Res 2016;22:197-202.
[27] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423-37.
[28] Kasashima H, Yashiro M, Kinoshita H, et al. Lysyl oxidase-like 2 (LOXL2) from stromal fibroblasts stimulates the progression of gastric cancer. Cancer Lett 2014;354:438-46.
[29] Kim GJ, Rhee H, Yoo JE, et al. Increased expression of CCN2, epithelial membrane antigen, and fibroblast activation protein in hepatocellular carcinoma with fibrous stroma showing aggressive behavior. PLoS One 2014;9:e105094.
[30] Porter S, Scott SD, Sassoon EM, et al. Dysregulated expression of adamalysin-thrombospondin genes in human breast carcinoma. Clin Cancer Res 2004;10:2429-40.