

Upregulated Expression of ADAM17 Is a Prognostic Marker for Patients With Gastric Cancer

Zhang-Xuan Shou, MM,* Xue Jin,* and Zhong-Sheng Zhao, PhD†

Objective: This study was designed to evaluate the expression and prognostic significance of A Disintegrin and Metalloproteinase 17 (ADAM17) protein in patients with gastric cancer.

Background: Tumor invasion and metastasis are primary causes for treatment failure or death among cancer patients. ADAM17 is a multidomain transmembrane glycoprotein involved in the release of several ligands that were shown to promote tumor formation and progression. Elevated expression of ADAM17 was detected in a number of human cancers and was associated with poor progression and prognosis of the diseases. In gastric cancer, however, the expression and prognostic significance of ADAM17 has not been fully elucidated.

Methods: The expressions of ADAM17 and extracellular matrix metalloproteinase inducer (EMMPRIN), a protein implicated in tumor invasion and metastasis, were detected using the tissue microarray technique and immunohistochemical EnVision method and compared with clinicopathological parameters of patients with gastric cancer.

Results: The expressions of ADAM17 and EMMPRIN were upregulated in gastric cancer lesions compared with their expressions in adjacent non-cancerous tissues ($P < 0.01$). High expression of ADAM17 was detected in 35.78% (156/436) of patients with gastric cancer and positively correlated with the expression of EMMPRIN ($r = 0.738$, $P < 0.01$). ADAM17 expression was associated with a number of clinicopathological parameters including depth of invasion and TNM stage of the tumor ($P < 0.05$). In each TNM stage, patients with high ADAM17 expression had a longer mean survival time than those with low expression ($P < 0.05$). Particularly, the mean survival time of stage II gastric cancer patients with low ADAM17 expression was longer than that of stage I patients with high ADAM17 expression ($P < 0.01$). Multivariate survival analysis suggested that, along with other parameters, ADAM17 and EMMPRIN expression were independent prognostic factors for patients with gastric cancer.

Conclusions: ADAM17 was implicated in the progression of gastric cancer. On the basis of the TNM stage, detection of ADAM17 expression will be helpful for predicting prognosis of gastric cancer.

Keywords: ADAM17, EMMPRIN, gastric cancer, immunohistochemistry, prognosis

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Gastric cancer is a major public health issue. It causes about 740,000 deaths worldwide per year, making it the second leading cause of cancer-related deaths after lung cancer.¹ Although the incidence of this disease has been declining, some 400,000 new cases are diagnosed every year in China, accounting for 42% of the world

total.² Gastric cancer is often asymptomatic or causes only nonspecific symptoms in its early stages. By the time symptoms occur, the cancer has often reached an advanced stage and this is one of the main reasons for its poor prognosis. Tumor invasion and metastasis are primary causes for treatment failure or death among cancer patients. They are enormously complex and multistep processes, involving regulation at the molecular level of adhesive molecules, proteolytic enzymes, and cell growth and angiogenesis factors.³ As a major member of the A Disintegrin and Metalloproteinase (ADAM) protein family, ADAM17 plays a pivotal role in the cleavage and activation of membrane-anchored receptor ligands. It has been revealed that ADAM17 is a potent sheddase of the epidermal growth factor family of ligands, which regulates the epidermal growth factor receptor activity in a variety of tumors.⁴ Epidermal growth factor receptor is a key component of autonomous growth signaling in several tumors, and its expression correlates with poor prognosis of gastric cancer patients.⁵ Elevated expression of ADAM17 was detected in a number of human cancers and was associated with poor progression^{6,7} and prognosis^{8,9} of the diseases. In gastric cancer, however, the expression and prognostic significance of ADAM17 has not been fully elucidated.

Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147, is a cell surface glycoprotein that belongs to the immunoglobulin superfamily. It is highly expressed on the surface of various malignant tumor cells and is involved in cancer progression.¹⁰ EMMPRIN not only plays a crucial role in degradation of the extracellular matrix through the induction of matrix metalloproteinase^{11,12} and the urokinase-type plasminogen activation system of serine proteases^{13,14} but also stimulates tumor angiogenesis by upregulating vascular endothelial growth factor expression in host cells and in the tumor cells themselves.^{15,16} In addition, EMMPRIN stimulates cell survival pathway signaling and confers multidrug resistance to some chemotherapeutic drugs in a hyaluronan-dependent manner.¹⁷ It was shown that the expression of EMMPRIN was upregulated in gastric cancer, and the increased expression of EMMPRIN possibly contributed to the genesis, growth, and local invasion of the cancer.¹⁸ Recently, the abnormally high level of EMMPRIN in cancer cells has been attributed to the dysregulation of epidermal growth factor receptor signaling.¹⁹ This result led us to speculate that ADAM17 may influence the expression of EMMPRIN in gastric cancer by activating epidermal growth factor receptor.

The aim of this study was to evaluate the clinical significance of ADAM17 in the progression and prognosis of gastric cancer, and to explore the potential association between ADAM17 and EMMPRIN in cancer progression. The expression of ADAM17 was detected and correlated with the EMMPRIN expression data and clinicopathological parameters of 436 patients with gastric cancer.

METHODS

Patients and Tissue Samples

After approval by the ethical committee of Zhejiang Provincial People's Hospital and written informed consent for use of the resected samples, 436 of 1200 consecutive patients who underwent gastrectomy for gastric cancer at Zhejiang Provincial People's

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Hospital between January 1998 and January 2004 were included in this retrospective study. All cases were diagnosed clinically at the Department of Gastrointestinal Surgery and histopathologically at the Department of Pathology. The patient cohort consisted of 311 males and 125 females, with a median age of 64 years (range: 30 to 91 years) at the time of surgery. The deadline for follow-up was December 2008. All patients had follow-up records for more than 5 years. The survival time was calculated from the date of surgery to the deadline for follow-up, or to the date of death. Among the 436 cases of gastric cancer, 55 were from the cardia, 163 from the body, and 218 from the antrum. According to the 2002 World Health Organization histological classification of gastric carcinoma, of the 436 cases, 16 were papillary, 326 tubular, 29 mucinous, and 65 signet-ring cell adenocarcinomas; 13 were highly differentiated, 128 well or moderately differentiated, 293 poorly differentiated, and 2 were undifferentiated adenocarcinomas. On the basis of Lauren's classification of gastric cancer, 223 cases were intestinal type and 213 were diffuse type. There were 61 cases with distant metastasis and 270 cases with lymph node metastasis. In terms of the International Union Against Cancer's, TNM classification system for gastric cancer, 90 cases were categorized as stage I, 104 as stage II, 173 as stage III, and 69 as stage IV. Following surgery, routine chemotherapy was given to patients with advanced disease; no radiation treatment was administered to any of the patients. Gastric cancer tissues were collected from the gastrectomy specimens of the 436 patients, and 92 non-cancerous gastric tissues were obtained from gastrectomy of adjacent gastric cancer margins greater than 5 cm. All tissues were formalin-fixed and paraffin-embedded samples.

Construction of Tissue Microarray

For diagnostic confirmation and to establish the representative area for each tumor tissue before constructing the tissue microarray (TMA), 4- μ m thick sections were cut from each tissue block and stained with hematoxylin and eosin (H&E). Subsequently, TMA blocks containing the gastric cancers and the non-cancerous gastric mucosae were prepared using the method described previously.²⁰ Briefly, tissue cylinders with a diameter of 2 mm were punched out from the targeted area of each donor tissue block and transferred into a recipient block using a TMA instrument; each TMA block contained 7 to 8 non-cancerous gastric mucosae as controls. Consecutive 4- μ m thick sections were then cut from each of the TMA blocks, and 1 section from each block was H&E stained for histological verification of the adequacy of the arrayed tumor tissues. Qualified samples were defined as those in which the tumor tissue occupied more than 10% of the core area. Sections were then placed on microscope slides for immunohistochemistry.

Immunohistochemistry

Immunohistochemical staining was performed on the TMA slides using the EnVision method as described previously.²¹ Briefly, the TMA slides were baked at 60°C for 2 hours, deparaffinized with xylene, and rehydrated in graded alcohol. Endogenous peroxidase was blocked with 3% H₂O₂ in methanol. The slides were then immersed in 0.01 M citrate buffer (pH 6.0) and microwaved for antigen retrieval and incubated with 10% normal goat serum at room temperature for 10 minutes to reduce nonspecific reactions. Subsequently, the slides were incubated overnight at 4°C with mouse monoclonal antibody to human ADAM17 (Abcam, Cambridge, UK) at a concentration of 3 μ g/mL and mouse monoclonal antibody to human EMMPRIN (Abcam, Cambridge, UK) at a concentration of 50 μ g/mL. After rinsing 3 times with 0.01 M phosphate buffer (pH = 7.2), the slides were incubated with secondary antibody (EnVision; DAKO, Glostrup, Denmark) for 20 minutes at room temperature and stained with diaminobenzidine (DAB)-H₂O₂. Finally, the TMA slides were

counterstained with hematoxylin, dehydrated, and mounted with a coverslip using a standard medium. Phosphate buffer was used to replace the primary antibody as a negative control.

Evaluation of Immunostaining Intensity

The ADAM17 and EMMPRIN proteins were immunohistochemically stained yellowish to brown in the cytoplasm and on the membrane of tumor cells. The immunoreactivity for each protein was reviewed under a light microscope by 2 pathologists who were blinded to the clinical data and scored independently according to the intensity of cellular staining and the proportion of stained tumor cells.²² In cases of discrepancy, a consensus score was chosen for evaluation. The staining intensity was scored as 0 (*no staining*), 1 (*weak staining, light yellow*), 2 (*moderate staining, yellow brown*), and 3 (*strong staining, brown*), and the proportion of stained tumor cells was classified as 0 ($\leq 5\%$ positive cells), 1 (6% to 25% positive cells), 2 (26% to 50% positive cells), and 3 ($\geq 51\%$ positive cells). The product of the scores for intensity and proportion was used to signify the level of protein expression, and a cut-off value was determined on the basis of a measure of heterogeneity using the log-rank test with respect to overall survival. The expression of both proteins was considered low if the product was 3 or less and high if the product was 4 or more.

Statistical Analysis

All statistical analyses were performed using SPSS 16.0 for Windows (SPSS, Chicago, IL). To assess the relationships between the expression of ADAM17 and EMMPRIN and the clinicopathological parameters of the patients with gastric cancer, the χ^2 test or Fisher exact test was used. Spearman rank correlation test was employed to evaluate the relationship between ADAM17 and EMMPRIN expression in gastric cancer. The cumulative survival rate was calculated using a life table method. Univariate survival analysis was performed using the Kaplan-Meier method, and the significance of difference between groups was analyzed with the log-rank test. Stepwise multivariate survival analysis was carried out using the Cox proportional hazards model. Variables that were significant in the univariate analysis were included in the model with the Backward Wald method. $P < 0.05$ was considered statistically significant and all P values were 2-sided.

RESULTS

Expression of EMMPRIN and ADAM17 in Gastric Cancer and Noncancerous Gastric Mucosa

The expression of ADAM17 protein was detected in 265 (60.78%) of the 436 patients with gastric cancer and negative in all 92 non-cancerous gastric mucosae. High expression of ADAM17 was detected in 156 (35.78%) cases, and low expression was detected in 109 (25.00%) cases. The immunostaining for ADAM17 was mainly located in the cytoplasm of the tumor cells (Fig. 1). The ADAM17 expression in gastric cancers was significantly higher than that in the non-cancerous gastric mucosae ($P < 0.01$). Similarly, the expression of EMMPRIN protein was detected in 290 (66.51%) of the 436 patients with gastric cancer and negative in all 92 non-cancerous gastric mucosae. High expression of EMMPRIN was detected in 161 (36.93%) cases, and low expression was detected in 129 (29.59%) cases. The immunostaining for EMMPRIN was dominantly distributed on the membranes of the tumor cells (Fig. 2). The EMMPRIN expression in gastric cancers was significantly higher than that in non-cancerous gastric mucosae ($P < 0.01$).

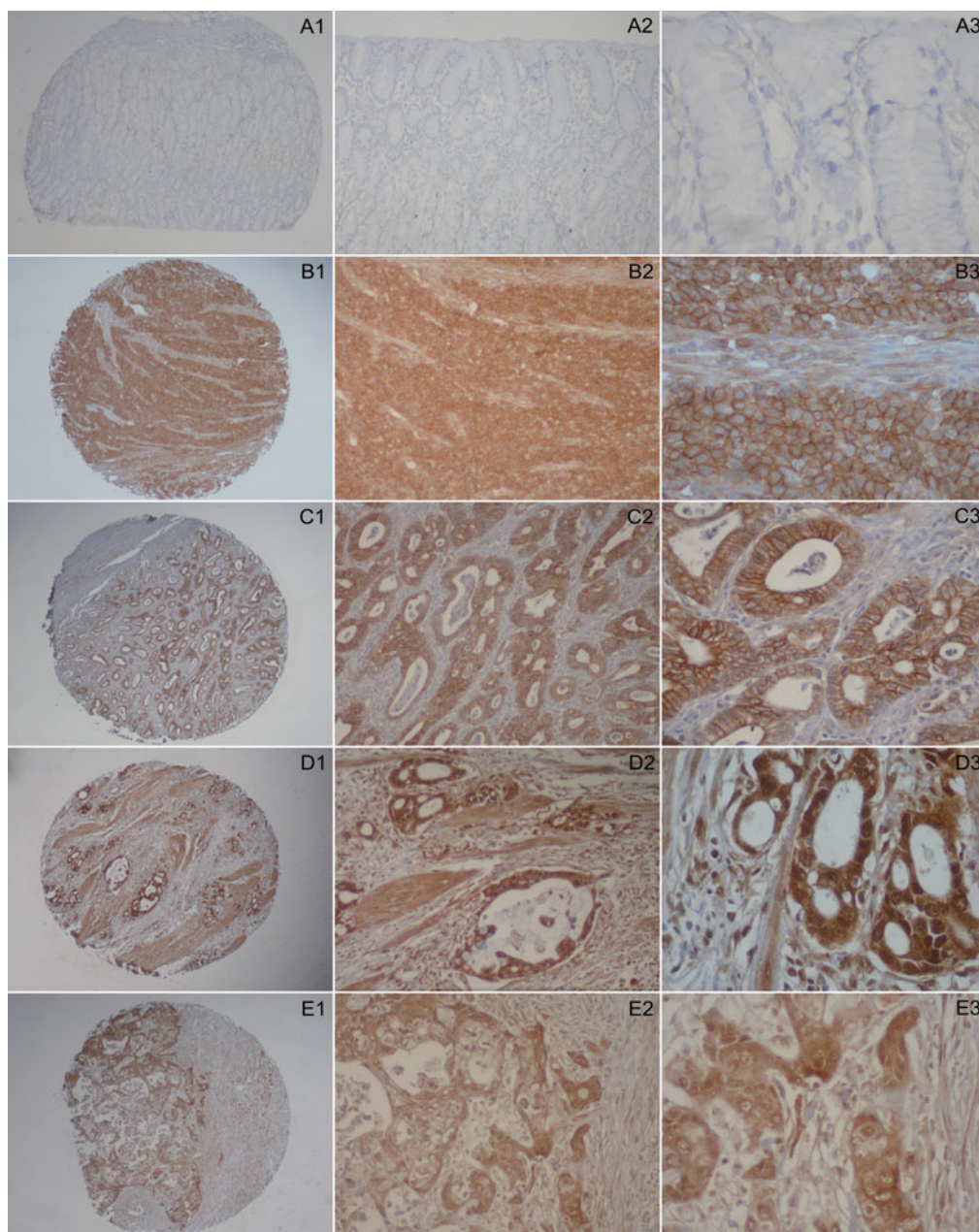


FIGURE 1. Immunohistochemical staining for ADAM17 in normal and cancerous gastric tissue (EnVision method). **A1 to A3**, no staining in non-cancerous gastric mucosa; **B1 to B3**, moderate staining in poorly differentiated adenocarcinoma; **C1 to C3**, strong staining in moderately differentiated adenocarcinoma; **D1 to D3**, strong staining in moderately differentiated adenocarcinoma; **E1 to E3**, moderate staining in poorly differentiated adenocarcinoma. Magnification: the original magnification $\times 40$ (**A1 to E1**), $\times 100$ (**A2 to E2**), and $\times 400$ (**A3 to E3**).

Correlation Between Expression of ADAM17 and EMMPRIN in Gastric Cancer

A high coincidental expression of the ADAM17 and EMMPRIN proteins was observed in gastric cancer. Of the 156 patients with high expression of ADAM17, 132 (84.62%) also had a high expression of EMMPRIN. The correlation between the expression of ADAM17 and EMMPRIN in patients with gastric cancer was statistically significant ($r = 0.738$, $P < 0.01$).

Association of ADAM17 and EMMPRIN Expression With Clinicopathological Parameters

The expression of ADAM17 in gastric cancer was significantly related to age, tumor size, depth of invasion, TNM stage, Lauren's classification, vessel invasion, lymph node metastasis, and distant metastasis of tumor, and not related to sex, location, differentiation, and histological type of the tumor. Gastric cancer patients with deep tumor invasion (T3 and T4), high TNM stage (stages III and IV),

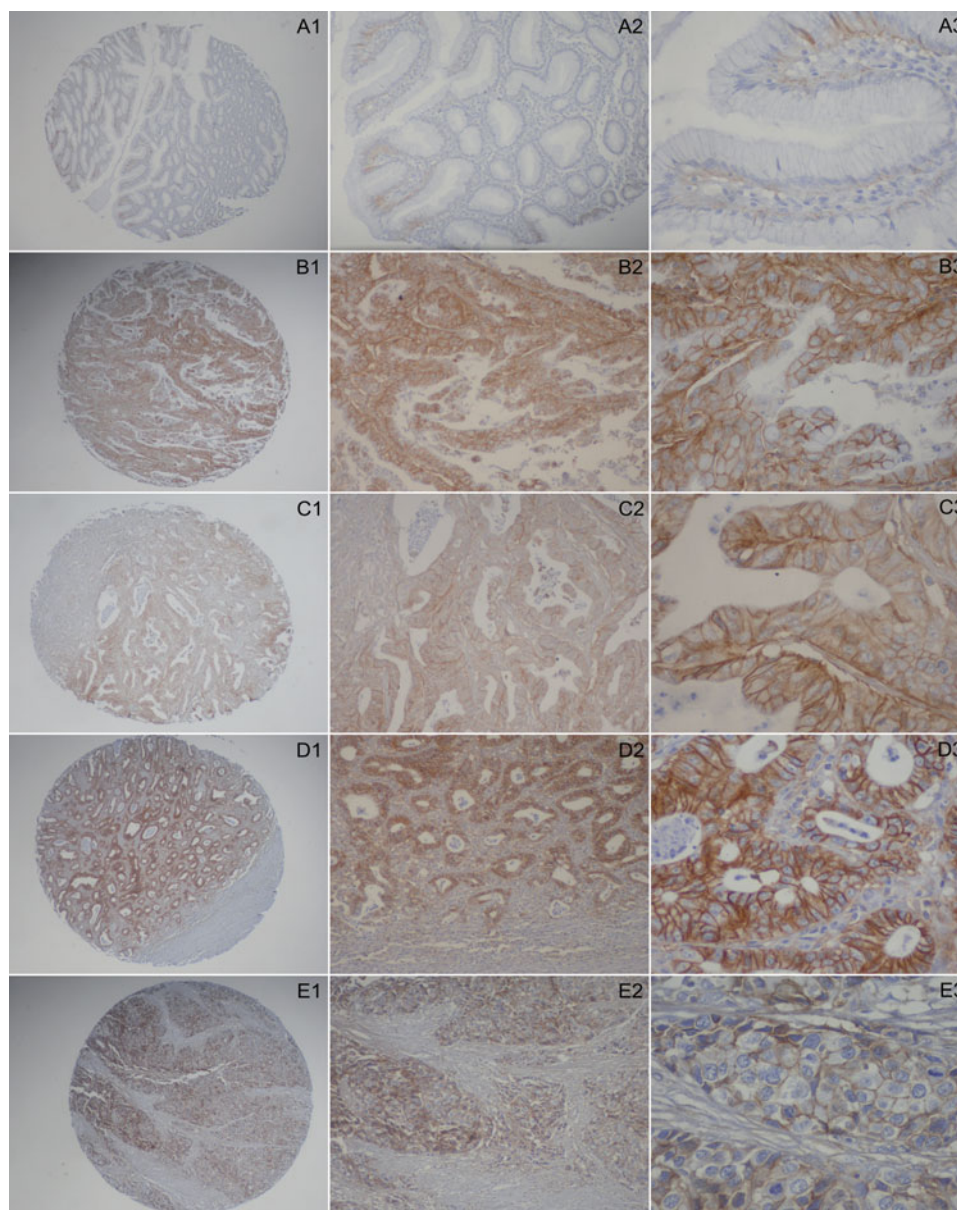


FIGURE 2. Immunohistochemical staining for EMMPRIN in normal and cancerous gastric tissue (EnVision method). **A1 to A3**, no staining in non-cancerous gastric mucosa; **B1 to B3**, strong staining in papillary adenocarcinoma; **C1 to C3**, moderate staining in moderately differentiated adenocarcinoma; **D1 to D3**, strong staining for EMMPRIN in well differentiated adenocarcinoma; **E1 to E3**, strong staining in poorly differentiated adenocarcinoma. Magnification: the original magnification $\times 40$ (**A1 to E1**), $\times 100$ (**A2 to E2**), and $\times 400$ (**A3 to E3**).

vessel invasion, lymph node metastasis, and distant metastasis had significantly higher expression of ADAM17 than those with superficial tumor invasion (T1 and T2), low TNM stage (stages I and II), and no vessel invasion, and lymph node and distant metastasis (Table 1). The Spearman correlation coefficient of ADAM17 expression with depth of invasion, TNM stage, vessel invasion, lymph node metastasis, and distant metastasis of tumor were 0.386, 0.590, 0.457, 0.483, and 0.268 ($P < 0.05$), respectively. Similarly, the expression of EMMPRIN in gastric cancer was significantly related to age, tumor size, location, depth of invasion, TNM stage, Lauren's classification, vessel invasion, and lymph node and distant metas-

tasis of tumor, and not related to sex, differentiation, and histological type of the tumor. Patients with deep tumor invasion (T3 and T4), high TNM stage (stages III and IV), vessel invasion, lymph node and distant metastasis had significantly higher expression of EMMPRIN than those with superficial tumor invasion (T1 and T2), low TNM stage (stages I and II), and no vessel invasion, lymph node and distant metastasis (Table 1). The Spearman correlation coefficient of ADAM17 expression with depth of invasion, TNM stage, vessel invasion, lymph node metastasis, and distant metastasis of tumor were 0.408, 0.599, 0.468, 0.504, and 0.323 ($P < 0.05$), respectively.

TABLE 1. Association of ADAM17 and EMMPRIN Expression With Clinicopathological Parameters of Patients With Gastric Cancer

Clinicopathological Parameters	Total	High ADAM17 Expression			High EMMPRIN Expression		
		n (%)	χ^2	P	n (%)	χ^2	P
Sex			0.892	0.345		1.129	0.288
Male	311	107 (34.4)			110 (35.4)		
Female	125	49 (39.2)			51 (40.8)		
Age range, yr			4.692	0.030		6.218	0.013
≤60	237	74 (31.2)			75 (31.6)		
>60	199	82 (41.2)			86 (43.2)		
Location of tumor			5.517	0.063		10.094	0.006
Cardia	55	25 (45.5)			29 (52.7)		
Body	163	64 (39.3)			65 (39.9)		
Antrum	218	67 (30.7)			67 (30.7)		
Tumor size, cm			31.359	0.000		30.794	0.000
<5	256	64 (25.0)			67 (26.2)		
≥5	180	92 (51.1)			94 (52.2)		
Depth of invasion			45.547	0.000		45.481	0.000
T1	57	3 (5.3)			8 (14.0)		
T2	109	27 (24.8)			25 (22.9)		
T3	244	110 (45.1)			108 (44.3)		
T4	26	16 (61.5)			20 (76.9)		
Vessel invasion			67.146	0.000		73.291	0.000
Negative	183	25 (13.7)			25 (13.7)		
Positive	253	131 (51.8)			136 (53.8)		
TNM stage			108.519	0.000		118.900	0.000
I	90	7 (7.8)			9 (10.0)		
II	104	17 (16.3)			15 (14.4)		
III	173	78 (45.1)			80 (46.2)		
IV	69	54 (78.3)			57 (82.6)		
Distant metastasis			37.191	0.000		66.353	0.000
Negative	375	113 (30.1)			110 (29.3)		
Positive	61	43 (70.5)			51 (83.6)		
Lymph node metastasis			62.408	0.000		61.261	0.000
Negative	166	21 (12.7)			23 (13.9)		
Positive	270	135 (50.0)			138 (51.1)		
Lauren's classification			162.549	0.000		184.097	0.000
Intestinal	223	16 (7.2)			14 (6.3)		
Diffuse	213	140 (65.7)			147 (69.0)		
Grade of differentiation			2.325	0.127		2.069	0.150
Well and moderate	141	44 (31.2)			46 (32.6)		
Poor and not	295	112 (38.0)			115 (39.0)		
Histological type			2.470	0.481		2.927	0.403
Papillary	16	5 (31.2)			6 (37.5)		
Tubular	326	111 (34.0)			114 (35.0)		
Mucinous	29	12 (41.4)			11 (37.9)		
Signet-ring cell	65	28 (43.1)			30 (46.2)		

Correlation of ADAM17 and EMMPRIN Expression With Prognosis

The 3- and 5-year cumulative survival rates were 90% and 55%, respectively, for patients with low ADAM17 expression, and 26% and 7%, respectively, for those with high ADAM17 expression. The mean survival time for patients with low expression of ADAM17 was 51.7 months, and 27.4 months for those with high expression of ADAM17. Clearly, gastric cancer patients with high expression of ADAM17 have a poorer prognosis than those with low ADAM17 expression ($P < 0.01$). Likewise, the 3- and 5-year cumulative survival rates were 91% and 57%, respectively, for gastric cancer patients with low expression of EMMPRIN and 26% and 6%, respectively, for those with high EMMPRIN expression. The mean survival time for patients with low expression of EMMPRIN in gastric cancer was 52.0 months, and 27.7 months for those with high expression of EMMPRIN. Thus, gastric cancer patients with high expression of EMMPRIN have

a poorer prognosis than those with low expression of EMMPRIN ($P < 0.01$). Univariate analysis indicated that the factors significantly associated with survival were age, tumor size, location, depth of invasion, TNM stage, Lauren's classification, vessel invasion, and lymph node and distant metastasis, whereas histological type and grade of differentiation were not related to the prognosis of the patients (Table 2). When stratified by TNM stage, it was found that, in each stage, the patients with low expression of ADAM17 had significantly longer mean survival times than those with high expression of ADAM17. Particularly, the mean survival time (56.8 months) for stage II gastric cancer patients with low expression of ADAM17 was significantly longer than 41.3 months for stage I patients with high expression of ADAM17 (Fig. 3). The clinicopathological parameters that were correlated with the survival of the patients in the univariate analysis were included in the multivariate analysis. Covariates included in the Cox regression model were age, tumor size, location,

TABLE 2. Univariate Analysis of the Correlation Between Clinicopathological Parameters and Survival of Patients With Gastric Cancer

Clinicopathological Parameters	Cumulative Survival Rates, %		Mean Survival Time, mo	Log-Rank Test	P
	3-Year	5-Year			
Age range, yr				14.745	0.000
≤60	74	44	45.85		
>60	59	29	39.63		
Location of tumor				7.849	0.020
Cardia	55	24	37.76		
Body	67	39	43.22		
Antrum	71	39	44.13		
Tumor size, cm				49.579	0.000
<5	78	49	47.50		
≥5	52	21	36.63		
Histological type				0.934	0.817
Papillary	69	24	41.92		
Tubular	67	39	43.26		
Mucinous	79	29	44.35		
Signet-ring cell	63	38	41.54		
Grade of differentiation				0.617	0.432
well and moderate	73	36	44.12		
poor and not	64	38	42.45		
TNM stage				370.398	0.000
I	96	94	58.09		
II	87	76	52.97		
III	61	7	37.70		
IV	16	1	23.26		
Depth of invasion				135.118	0.000
T1	93	91	57.18		
T2	82	62	50.01		
T3	58	18	38.38		
T4	35	8	26.85		
Lymph node metastasis				176.051	0.000
Negative	88	82	54.23		
Positive	54	12	36.30		
Distant metastasis				141.372	0.000
Negative	75	43	46.23		
Positive	29	3	23.18		
Vessel invasion				127.410	0.000
Negative	90	70	52.56		
Positive	51	16	36.26		
Lauren's classification				239.586	0.000
Intestinal	93	66	54.12		
Diffuse	40	9	31.56		
EMMPRIN expression				274.677	0.000
Low	91	57	51.99		
High	26	6	27.72		
ADAM17 expression				259.864	0.000
Low	90	55	51.71		
High	26	7	27.45		

depth of invasion, TNM stage, Lauren's classification, vessel invasion, lymph node metastasis, distant metastasis, ADAM17 expression, and EMMPRIN expression. It was indicated that age, Lauren's classification, TNM stage, depth of invasion, lymph node metastasis, distant metastasis, ADAM17 expression, and EMMPRIN expression were independent prognostic factors for patients with gastric cancer, whereas tumor size, location, and vessel invasion were not (Table 3).

DISCUSSION

Since its discovery as the tumor necrosis factor- α converting enzyme, ADAM17 has been widely studied. It has been shown to be an indispensable regulator of almost every cellular event from proliferation to migration. In this retrospective study, we evaluated the expression of ADAM17 in gastric cancer and its prognostic im-

plications. The expression of ADAM17 was upregulated in gastric cancers as compared with that in adjacent non-cancerous gastric mucosae, and elevated ADAM17 expression was significantly associated with age, tumor size, TNM stage, depth of invasion, Lauren's classification, vessel invasion, and lymph node and distant metastasis. ADAM17 expression was highest in the tumors at high TNM stages (stages III and IV), with deep invasion (T3 and T4), presence of vessel invasion, and lymph node and distant metastasis, indicating that ADAM17 overexpression was involved in the progression of gastric cancer.

ADAM17 has been implicated in carcinogenesis because it was shown to contribute to the inflammation often observed in tumors, and because it sheds the growth factors necessary for tumor progression and growth.²³ The ADAM17-catalyzed shedding of

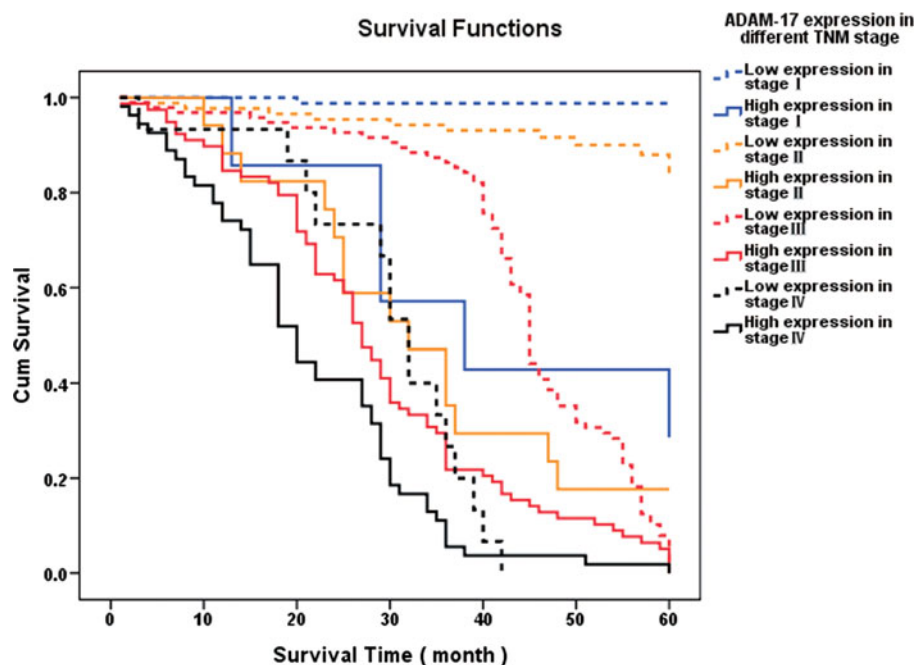


FIGURE 3. Kaplan–Meier survival curves of gastric cancer patients with different level of ADAM17 expression stratified by the TNM stage of the tumor (log-rank test). Stage I gastric cancers with low ADAM17 expression versus high ADAM17 expression, log rank = 58.122, $P = 0.000$; stage II gastric cancers with low ADAM17 expression versus high ADAM17 expression, log rank = 55.102, $P = 0.000$; stage III gastric cancers with low ADAM17 expression versus high ADAM17 expression, log rank = 34.181, $P = 0.000$; stage IV gastric cancers with low ADAM17 expression versus high ADAM17 expression, log rank = 4.835, $P = 0.028$; stage II gastric cancers with low ADAM17 expression versus stage I gastric cancers with high ADAM17 expression, log rank = 17.586, $P = 0.000$.

TABLE 3. Multivariate Analysis of the Correlation Between Clinicopathological Parameters and Survival Time of Patients With Gastric Cancer

Covariates	Coefficient	Standard Error	HR	95% CI for HR	P
Age range (>60 vs ≤60 yr)	0.379	0.131	1.458	1.130–1.888	0.004
Tumor location (cardia vs others)	0.031	0.179	1.034	0.726–1.466	0.861
Tumor size (≥5 vs <5 cm)	0.044	0.131	1.045	0.808–1.350	0.740
Lauren's classification (diffuse vs intestinal)	0.661	0.172	1.927	1.384–2.712	0.000
Lymph node metastasis (positive vs negative)	0.752	0.295	2.072	1.190–3.781	0.011
Vessel invasion (positive vs negative)	-0.240	0.187	0.782	0.545–1.136	0.201
Distant metastasis (positive vs negative)	0.659	0.166	1.933	1.395–2.679	0.000
EMMPRIN expression (high vs low)	0.452	0.178	1.572	1.108–2.228	0.011
ADAM17 expression (high vs low)	0.724	0.171	2.067	1.475–2.883	0.000
TNM stage (stage III and IV vs I and II)	1.084	0.341	3.045	1.515–5.774	0.001
Depth of invasion (T3, T4 vs T1, T2)	0.651	0.176	1.915	1.358–2.708	0.000

CI indicates confidence interval; HR, hazard ratio.

transforming growth factor α , amphiregulin, heparin-binding epidermal growth factor-like growth factor and epiregulin has been reported to mediate tumor formation and progression.^{24–27} ADAM17 induces proliferation, migration, and tube formation in breast cancer cells by activating the epidermal growth factor receptor-phosphoinositide 3-kinases-AKT pathway.²⁸ Aberrant expression of ADAM17 increased the malignant potential of tumors in human pancreatic ductal adenocarcinoma.²⁹ ADAM17 expression was significantly enhanced in both early and advanced ovarian cancer compared with that in normal ovaries.³⁰ In addition, high levels of the ADAM17 transcript were detected in gastric cancer,³¹ suggesting that ADAM17 may play a crucial role in the formation and progression of tumors through ectodomain shedding of the ligands for epidermal growth factor receptor.³²

Until now, invasion depth, TNM stage, and lymph node and distant metastasis have been considered to be the prognostic factors for gastric cancer.³³ In this study, we found that high expression of ADAM17 was significantly associated with poor prognosis of patients with gastric cancer, and that the expression of ADAM17 was an inde-

pendent prognostic factor. Other factors significantly correlated with the survival of the patients included age, tumor size, location, depth of invasion, TNM stage, Lauren's classification, vessel invasion, lymph node metastasis, distant metastasis, and EMMPRIN expression. Further analysis revealed that patients with low expression of ADAM17 had a significantly longer mean survival time than those with high expression of ADAM17 in each TNM stage. In particular, the stage II patients with low expression of ADAM17 had a significantly longer mean survival time than the stage I patients with high expression of ADAM17, suggesting that ADAM17 can serve as an objective and effective indicator for the identification of gastric cancer patients that are at high risk for tumor invasion and progression. Our findings are in agreement with earlier reports that correlated elevated expression of ADAM17 with the poor progression and prognosis in many tumors. For example, in breast cancer⁸ and gallbladder carcinoma,⁹ the expression of ADAM17 significantly increased in high-grade tumors compared with low-grade tumors. Patients with high expression of ADAM17 had a significantly shorter overall survival time than those with low expression, and the prognostic effect of ADAM17

expression was independent of conventional prognostic factors for both tumors. For squamous cell carcinomas of the head and neck, the expression of ADAM17 messenger ribonucleic acid was associated with tumor stage and regional lymph node metastasis, patients expressing high levels of ADAM17 showed significantly reduced overall survival compared with those with low messenger ribonucleic acid levels.³⁴

In addition to being a potential prognostic factor for many malignancies, ADAM17 has also received considerable attention as an effective marker for predicting therapeutic outcomes and is a potential target for anti-cancer therapy. ADAM17 may have a role in identifying patients likely to be resistant to therapies directed against the epidermal growth factor receptor and HER-2. It was found that many patients with *HER2* gene-amplified metastatic breast cancers do not respond or eventually escape trastuzumab, a monoclonal antibody that interferes with the *HER2* neu receptor. Although multiple mechanisms are likely to be responsible for resistance to trastuzumab, one is the excessive formation of ligands that activate the epidermal growth factor receptor and promote heterodimerization with HER2.³⁵ Trastuzumab-resistant human breast cancer cells were found to express a number of epidermal growth factor receptor ligands, and one of these factors, transforming growth factor α , could completely negate the growth inhibitory effects of trastuzumab.³⁶ In non-small-cell lung cancer, excessive production of transforming growth factor α as well as amphiregulin has been shown to confer resistance to gefitinib,^{37,38} a tyrosine kinase inhibitor against the epidermal growth factor receptor. A selective ADAM inhibitor, INCB3619, prevented the processing and activation of multiple ErbB ligands, thereby inhibiting gefitinib-resistant HER3 signaling and enhancing gefitinib's inhibition of epidermal growth factor receptor signaling.³⁹ In colorectal cancer, chemotherapy with fluorouracil (5-FU) acutely activates ADAM17, which results in growth factor shedding, growth factor receptor activation, and drug resistance.⁴⁰ These observations indicate that ADAM17 inhibition affects multiple ErbB pathways, and thus offers an excellent opportunity for pharmacological intervention of human cancers. ADAM17 inhibitors would be expected to be particularly useful in tumors that are dependent on the epidermal growth factor receptor/HER-2 signaling or that have excessive ligand formation.

We found in our study that patients with high expression of ADAM17 also had a high rate of upregulated expression of EMMPRIN in gastric cancer, and the expression of ADAM17 positively correlated with that of EMMPRIN. Furthermore, the expression of EMMPRIN was significantly correlated with tumor size, depth of invasion, vessel invasion, TNM stage, distant metastasis, and lymph node metastasis, all of which are related to tumor progression. Overexpression of EMMPRIN may contribute to the genesis, growth, and local invasion of gastric cancer by upregulating matrix metalloproteinase expression in both stromal fibroblasts and cancer cells.¹⁸ The expression of the epidermal growth factor receptor was positively correlated with that of EMMPRIN in colorectal cancer.⁴¹ Treatment of growth factor-starved oral intraepithelial neoplasia cells with amphiregulin enhanced EMMPRIN messenger ribonucleic acid and protein expression and stimulated their growth in a dose and time-dependent fashion.⁴² Two ligands for the epidermal growth factor receptor, epidermal growth factor and amphiregulin, induced the expression of EMMPRIN in mammary tumor cells.⁴³ The induction of EMMPRIN by amphiregulin was mediated by the epidermal growth factor receptor's tyrosine kinase activation and inhibited by ZD1839, a specific tyrosine kinase inhibitor against the epidermal growth factor receptor; amphiregulin and epidermal growth factor receptor antisense complementary DNAs inhibited EMMPRIN expression. On the basis of these findings, we propose that the upregulated expression of ADAM17 may contribute to the production of EMMPRIN

through activation of the epidermal growth factor receptor and that the cross-talk between ADAM17 and EMMPRIN is likely to play a role in the mechanisms underlying carcinogenesis, development, and progression of gastric cancer.

Because of the subjective nature of scoring method used in our study, further study is needed to reevaluate the immunoreactivity of both proteins by another team of 2 pathologists independently, thereby certifying the reproducibility of immunohistochemical scores among evaluators. Moreover, before these proteins can be introduced as clinical biomarkers, standardization and quality control in immunohistochemical procedures and evaluation are necessitated to rule out bias owing to differences in tissue processing and in laboratory protocols for staining and evaluation by different observers.⁴⁴

CONCLUSION

It was demonstrated in our study that the expression of ADAM17 was upregulated in gastric cancer, high expression of ADAM17 significantly correlated with poor prognosis of patients with gastric cancer. Thus, ADAM17 may play an important role in the progression of gastric cancer and could be an objective and effective indicator to predict local invasion and prognosis of gastric cancer. On the basis of the TNM stage of the tumor, the expression of ADAM17 in gastric cancer will help identify patients with high potential for tumor invasion and metastasis, thereby guiding the clinical decision. In addition, our results provided further impetus for exploiting ADAM17 as a new target for gastric cancer therapy.

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