S100 calcium-binding protein A4 is a novel independent prognostic factor for the poor prognosis of gastric carcinomas

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Abstract. Overexpression of the S100 calcium-binding protein A4 (S100A4) is involved in epithelial-to-mesenchymal transition, oncogenic transformation, angiogenesis, cytoskeletal integrity and cancer metastasis. Here, we elucidated the role of S100A4 in tumorigenesis and progression of gastric carcinomas. S100A4 expression in gastric carcinomas, adenomas and adjacent non-neoplastic mucosa was analyzed by immunohistochemical, real-time reverse transcriptase (RT)-polymerase chain reaction (PCR) and western blot analyses, and was correlated with various clinicopathological parameters. S100A4 protein expression was increased gradually in the following order: gastritis (19.2%), intestinal metaplasia (IM; 23.3%), dysplasia (34.9%) and carcinoma (55.2%; $P<0.001$). S100A4 was positively correlated with tumor size, depth of invasion, lymphatic invasion, lymph node metastasis and tumor-node-metastasis (TNM) staging ($P<0.05$), but not with the age and gender of the carcinoma patients ($P>0.05$). Intestinal-type (IT) carcinomas showed a higher S100A4 expression than diffuse-type (DT) carcinomas ($P<0.001$). S100A4 mRNA expression also increased in the following order: gastritis < IM < dysplasia < carcinoma ($P<0.05$). S100A4 overexpression was observed in gastric carcinomas with a larger diameter, deeper invasion, lymph node metastasis and in IT carcinoma ($P<0.05$). Univariate analysis using the Kaplan-Meier method indicated a lower cumulative survival rate for patients with weak or moderate S100A4 expression compared with patients not expressing S100A4 ($P<0.001$). Multivariate analysis using Cox's proportional hazard model demonstrated that depth of invasion, lymphatic or venous invasion, lymph node metastasis, TNM staging and S100A4 expression were independent factors for poor patient prognosis ($P<0.05$). In conclusion, S100A4 upregulation is positively associated with the pathogenesis, growth, invasion, metastasis and differentiation of gastric carcinomas. S100A4 may be a promising marker indicative of the aggressive behavior and prognosis of gastric carcinomas.

Introduction

Gastric carcinoma is the second leading cause of cancer-related mortality in the world (1), after lung cancer. Despite a sharp worldwide decline in both the incidence and mortality of gastric carcinoma since the second half of the 20th century, it still continues to be a major health problem worldwide. This can be attributed to the particularly slow decrease in the incidence of gastric carcinoma in Asia and a high mortality rate of the diagnosed gastric carcinoma cases in the West (2). Therefore, research efforts that elucidate the molecular mechanisms underlying gastric carcinogenesis and subsequent disease progression and that identify reliable biomarkers are of critical importance for the prevention, treatment, and prognostic evaluation of gastric cancer.

The S100 proteins are calcium-binding, acidic, low-molecular-weight proteins (10-12 kDa), which are named according to their solubility in 100% saturated ammonium sulfate. In recent years, these proteins are gaining importance due to their association with diseases such as cardiomyopathy, neurodegenerative disorders and cancer (3). The S100 protein family includes ~25 members, with the genes clustered on human chromosome 1q21. The encoded proteins contain two EF-hand calcium-binding helix-loop-helix motifs. Each member contains two EF-hands connected by a central hinge, which is S100 family-specific in the N-terminus and canonical in C-terminus (4). Overexpression of the S100 calcium-binding protein A4 [S100A4, also known as Mts1 (metastasis-associated gene), p9Ka, 18A2, pEL98, 42A, CAPL and calvasculin] of the S100 protein family has been shown to control cell cycle progression and modulate intercellular adhesion, as well as invasive and metastatic properties of cancer cells (5). The human S100A4 gene is located at position 1q21 on chromosome 1 and encodes a polypeptide of 101 amino acids with a molecular mass of ~11.5 kDa (6). The S100A4 gene contains four exons, and its open reading frame begins from the third exon. The S100A4 promoter contains an ErbB2 response element, and the enhancer and silencer elements of the S100A4 gene may be strongly affected by methylation (7,8).

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The S100A4 protein has been shown to be present at detectable limits in a subset of cells of the ovary, prostate, spleen, thymus, bone marrow, T lymphocytes, neutrophils and macrophages, whereas very low levels have been detected in normal tissues of the pancreas, colon, thyroid, lungs and kidneys (8). S100A4 overexpression has been reported to be positively correlated with rheumatoid arthritis, kidney fibrosis, liver fibrosis, peritoneal fibrosis, corneal dystrophy, neural diseases, and cardiac and lung disease (7,8). Furthermore, S100A4 overexpression is involved in epithelial-to-mesenchymal transition (EMT), oncogenic transformation, and aggressive progression of cancers (9,10). Transgenic mice that overexpress S100A4 in the mammary epithelium are phenotypically indistinguishable from wild-type mice and exhibit increased metastasis (11). Inhibition of S100A4 (by antisense or anti-ribozyme approaches) suppressed the metastatic potential of tumor cells in animal models of lung carcinoma and osteosarcoma (12,13). Several proteins have been identified as targets for the S100A4 protein, including liprin β1 and methionine aminopeptidase (14). S100A4 is reported to bind to the C-terminal regulatory domain of p53 *in vitro* and inhibit its phosphorylation, thus suppressing the transcription of p53 target genes including p21/WAF, Bax, thrombospondin-1 and Mdm-2 (15). S100A4 is also known to interact with proteins involved in cytoskeletal rearrangement and cell motility such as F-actin, myosin-IIA, tropomyosin, and the heavy chain of nonmuscle myosin II (16-18). Additionally, S100A4 has been shown to exhibit its prometastatic role through its influence on angiogenesis, cytoskeletal integrity, matrix metalloproteinases (MMPs), tumor-related transcription factors and stromal factors (19,20).

The aim of the present study was to analyze S100A4 expression in gastric cancer and precancerous lesions at both the protein and mRNA levels and to further compare this S100A4 expression with the clinicopathological features of gastric cancer.

Materials and methods

Patients and tissue specimens. Gastric cancer tissue was collected from gastrectomy specimens and from gastritis, gastric intestinal metaplasia (IM), and adenoma specimens by endoscopic biopsies performed between January 1995 and January 2005 at Department of General Surgery, Shengjing Hospital of China Medical University. The tissues were fixed in 10% neutral formalin, embedded in paraffin, and cut into 4-μm sections. The sections were stained by hematoxylin and eosin (H&E) to confirm histological diagnosis and other microscopic characteristics. Portions of a few samples were frozen in liquid nitrogen until the tissue was homogenized for RNA and protein extraction. The tumor-node-metastasis (TNM) staging for each gastric carcinoma specimen was performed according to the Union Internationale Contre le Cancer (UICC) system for the extent of tumor spread (21). Histological architecture of the gastric carcinoma was expressed in terms of Lauren's classification (22,23). Furthermore, tumor size, depth of invasion, and lymphatic and venous invasion were determined. Lymphatic and venous invasion were assessed first by H&E staining, followed by D2-40 immunostaining (for lymphatic invasion) and EvG staining (for venous invasion), if necessary. None of the patients underwent chemotherapy, radiotherapy, or adjuvant treatment before surgery. The University Ethics Committee of China Medical University approved the research protocol. The patients were carefully followed up by consulting their case documents and through telephone monitoring.

Tissue microarray (TMA) and immunohistochemistry. Representative areas of the solid tumors were identified in the H&E-stained sections of the selected tumor cases, and a tissue core (2 mm in diameter) per donor block was punched out and transferred to a recipient block with a maximum of 48 cores using a manual arraying device (MTA-1; Beecher Instruments, Inc., Sun Prairie, WI, USA). Sections (4-μm) were consecutively incised from the recipient block and transferred to poly-lysine-coated glass slides. H&E staining was performed on the TMA sections for confirmation of the tumor tissue.

Immunohistochemistry was performed on 4-μm formalin-fixed, paraffin-embedded tissue sections. The sections were first deparaffinized in xylene and rehydrated through graded concentrations of alcohol. For antigen retrieval, endogenous peroxidase activity was blocked by incubating the slides in 1.5% hydrogen peroxide in absolute methanol at room temperature for 10 min. Next, the sections were subjected to antigen retrieval by heating the slides in target retrieval solution (TRS; Dako) for 15 min in a microwave oven (Oriental Rotor Ltd. Co., Tokyo, Japan). The rabbit polyclonal antibody against S100A4 (Abcam, Cambridge, UK; 1:200) was applied for 1 h at room temperature, according to the manufacturer's instructions, and the sections were washed three times with TBST. After a final rinse with TBST, the sections were further incubated with an anti-rabbit IgG antibody conjugated to horseradish peroxidase (Dako) at a dilution of 1:1,000 for 1 h. The final color was visualized by exposing the sections to 0.5 mg/ml 3,3'-diaminobenzidine and 0.005% hydrogen peroxide for ~5 min. After counterstaining with Mayer's hematoxylin, the sections were dehydrated, cleared and mounted. Sections without the addition of the primary antibody were used as a negative control.

The slides were randomly allocated for analysis by two independent experienced pathologists with no knowledge of the clinicopathological data. For each slide, the number of S100A4-positive cells was counted in 10 fields at x200 magnification, and the percentage of positively stained cells was determined. The percentage of positively stained cells was graded semiquantitatively according to a four-point scoring system as follows: negative (-), 0-5%; weakly positive (+), 6-25%; moderately positive (++), 26-50%; and strongly positive (+++), >50%.

Real-time reverse transcriptase (RT)-polymerase chain reaction (PCR). Total RNA was extracted using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), and cDNAs were reverse transcribed by the RevertAid™ RT (Takara Bio, Inc., Otsu, Shiga, Japan). Real-time PCR was carried out using the ABI PRISM® 7500 Sequence detection system (Applied Biosystems, Foster City, CA, USA) at 50°C for 2 min, 95°C for 10 min, followed by 50 cycles at 95°C for 15 sec, and at 60°C for 1 min. The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 135 bp, 201-335, NM_002046.3) were 5'-CAATGACCCCTTCATTGACC-3' (sense) and 5'-TGGAAGATGGTGATGGGATT-3' (antisense). The primers for S100A4 (130 bp, 248-377, NM_019554.2) were 5'-AGCTTCTTGGGGAAAAGGAC-3' (sense) and 5'-TACACATCATGG
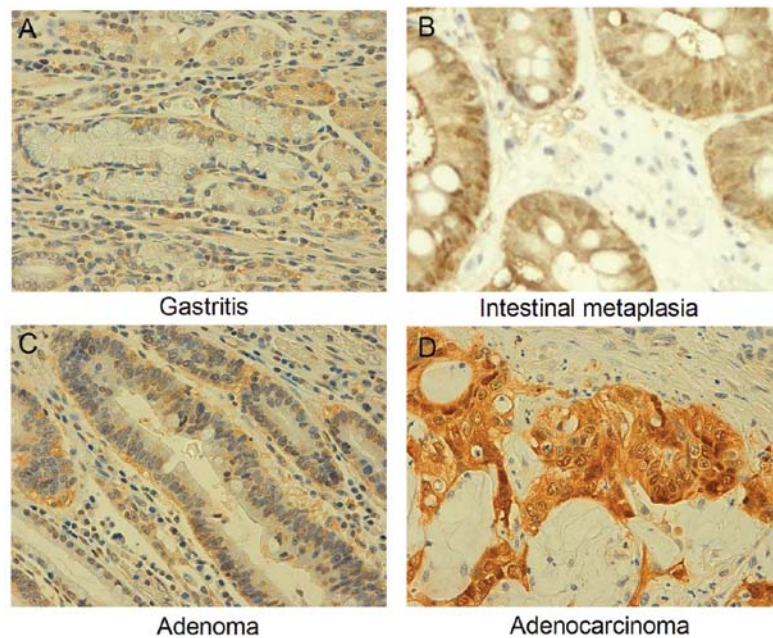
Figure 1. Immunohistochemical staining of the S100A4 protein in gastric lesions. The S100A4 protein was positively expressed in the cytoplasm of cells obtained from (A) gastritis, (B) intestinal metaplasia, (C) adenomatous dysplasia and (D) carcinoma specimens (magnification, x100).

CGATGCAG-3' (antisense). Expression of GAPDH was used to normalize expression of the target genes. Each assay was performed in triplicate, the average was calculated, and the expression level of S100A4 was expressed as $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct\,(S100A4) - Ct\,(GAPDH)$. $\Delta\Delta Ct = \Delta Ct\,(carcinoma) - \Delta Ct$ (mucosa).

Western blot analysis. Protein was extracted by homogenizing the tissues in RIPA lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 5 mM dithiothreitol, 10 mM NaF, protease inhibitor cocktail] (Sigma, St. Louis, MO, USA), and the amount of protein was concentrated by the bicinchoninic acid (BCA) method. The denatured proteins were separated on a 15% SDS-polyacrylamide gel and transferred to a Hybond-P PVDF membrane (Amersham plc, Amersham, Germany), which was then blocked overnight in 5% skim milk in TBST (10 mmol/l Tris-HCl, 150 mmol/l NaCl, 0.1% Tween-20). For immunoblotting, the membrane was incubated for 1 h with the rabbit antibody against S100A4 (1: 500). The membrane was rinsed with TBST and incubated with an anti-rabbit or anti-mouse secondary IgG conjugated to horseradish peroxidase (1:1,000; Dako, Carpinteria, CA, USA) for 1 h. Bands were visualized on X-ray film (Fuji, Japan) using the ECL Plus detection reagents (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Next, the membrane was washed with the WB Stripping Solution (pH 2.0-3.0; Nacalai, Tokyo, Japan) for 1 h and treated as described above, except an anti-GAPDH antibody (1:10,000; Sigma) was used as an internal loading control. Densitometric quantification of the S100A4 protein in gastric samples was performed using on Scion Image software (Scion Corporation, Frederick, MD, USA) using GAPDH as a control.

Statistical analyses. All quantitative data are expressed as means ± standard deviation (SD). The Student's t-test and one-way analysis of variance (ANOVA) with the Student's-Newman-Keuls t-test were used to compare the means of different groups. Categorical data were analyzed by the Spearman's correlation test. Kaplan-Meier survival plots were generated, and comparisons between survival curves were carried out with log-rank statistics. Cox's proportional hazards model was employed for multivariate analysis. SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze all data, and P<0.05 was considered to indicate a statistically significant result.

Results

Immunohistochemical analysis for S100A4 expression in gastric carcinomas. The expression of the S100A4 protein was assessed by determining the number of positively stained tumor cells using a four-point scoring system, as described in Materials and methods. As shown in Fig. 1, S100A4 was positively immunostained in the cytoplasm and nuclei of gastric epithelial cells, IM, adenomatous dysplasia, and carcinoma cells. Overall, S100A4 expression was detected in 19.2% of the gastritis cases (14/73), 23.3% of the IM cases (20/86), 34.9% of the adenomatous dysplasia cases (22/63), and 55.2% of the total gastric carcinoma cases (192/348). Based on the S100A4 expression frequency and density, we inferred that there was a gradual increase in S100A4 expression in the following order: gastritis < IM < dysplasia < carcinoma (P<0.001) (Table I).

Correlation between S100A4 expression and clinicopathological factors. Next, we analyzed the correlation between positive S100A4 expression and various clinicopathological factors that may affect the prognosis of patients with gastric carcinoma. As summarized in Table II, S100A4 expression was positively correlated with tumor size, depth of invasion,

Table I. S100A4 expression in gastric carcinogenesis.

Groups	N	S100A4 expression				PR (%)	P-value
		-	+	++	+++		
Gastritis	73	59	8	4	2	19.2	<0.001
IM	86	66	13	5	2	23.3	
Adenoma	63	41	13	7	2	34.9	
Gastric carcinoma	348	156	29	62	101	55.2	

S100A4, S100 calcium-binding protein A4; PR, positive rate; IM, intestinal metaplasia.

Table II. Relationship between S100A4 expression and clinicopathological features of the gastric carcinoma cases.

Clinicopathological features	N	S100A4 expression				PR (%)	P-value
		-	+	++	+++		
Age (years)							0.277
<55	139	67	12	23	37	51.8	
≥55	209	89	17	39	64	57.4	
Gender							0.183
Male	248	102	27	46	73	58.9	
Female	100	54	2	16	28	46.0	
Tumor size (cm)							<0.001
≤4	120	84	4	11	21	30.0	
>4	228	72	25	51	80	68.4	
Depth of invasion							<0.001
Tis-T1	33	25	2	2	4	24.2	
T2-T4	315	131	27	60	97	58.4	
Lymphatic invasion							<0.001
-	192	116	14	20	42	39.6	
+	156	40	15	42	59	74.4	
Venous invasion							0.44
-	245	135	17	32	61	44.9	
+	103	21	12	30	40	79.6	
LN							0.002
-	138	83	4	12	39	39.8	
+	210	73	25	50	62	65.2	
TNM staging							<0.001
I	6	6	0	0	0	0.0	
II	60	48	2	4	6	20.0	
III	104	50	5	15	34	51.9	
IV	178	52	22	43	61	70.8	
Lauren's classification							<0.001
IT	203	131	7	23	42	35.5	
DT	145	25	22	39	59	82.8	

S100A4, S100 calcium-binding protein A4; PR, positive rate; Tis, carcinoma *in situ*; T1, lamina propria and submucosa; T2, muscularis propria and subserosa; T3, exposure to serosa; T4, invasion into serosa; TNM, tumor-node-metastasis LN, lymph node metastasis; IT, intestinal-type; DT, diffuse-type.

lymphatic and venous invasion, lymph node metastasis, and TNM staging (all P<0.05) in carcinoma patients. Age and gender, however, had no influence on S100A4 expression in the carcinoma patients (P>0.05). S100A4 expression was higher in intestinal-type (IT) carcinomas than that in diffuse-type (DT) carcinomas (P<0.001).
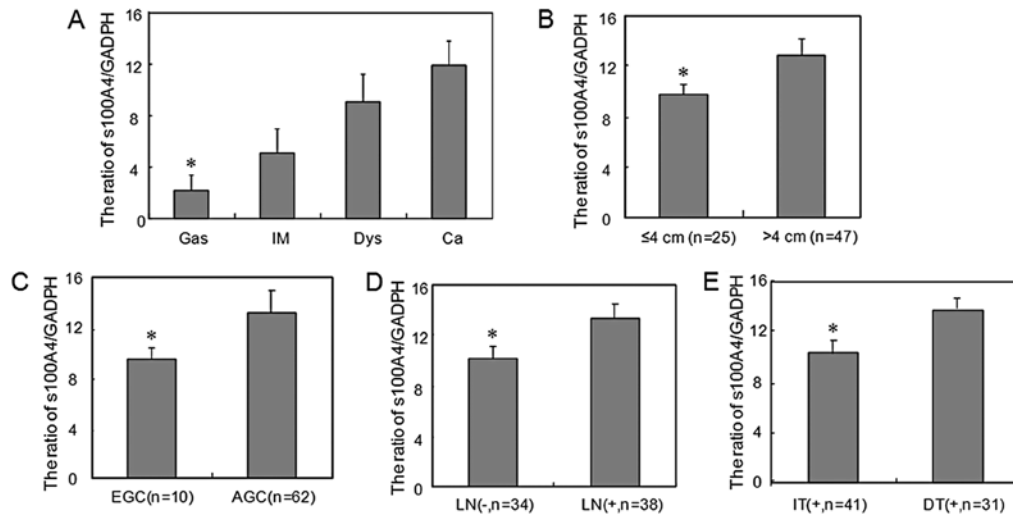
Figure 2. S100A4 mRNA expression level during gastric carcinogenesis and its correlation with clinicopathological features of carcinoma. (A) S100A4 mRNA was detectable in gastritis (Gas), intestinal metaplasia (IM), dysplasia (Dys), and carcinoma (Ca) samples, as revealed by real-time RT-PCR analysis, and it gradually increased from Gas, IM, Dys to Ca (*P<0.05; Ca vs. Gas). S100A4 mRNA expression was higher in gastric carcinomas that showed (B) a larger diameter (*P<0.05; >4 cm vs. ≤4 cm), (C) deeper invasion (*P<0.05; AGC vs. EGC), (D) frequent lymph node metastasis [*P<0.05; LN(+) vs. ≤LN(-)], and (E) intestinal-type (*P<0.05; DT vs. IT). Data are presented as means ± SD. EGC, early gastric cancer; AGC, advanced gastric cancer; LN, lymph node metastasis; IT, intestinal-type; DT, diffuse-type.
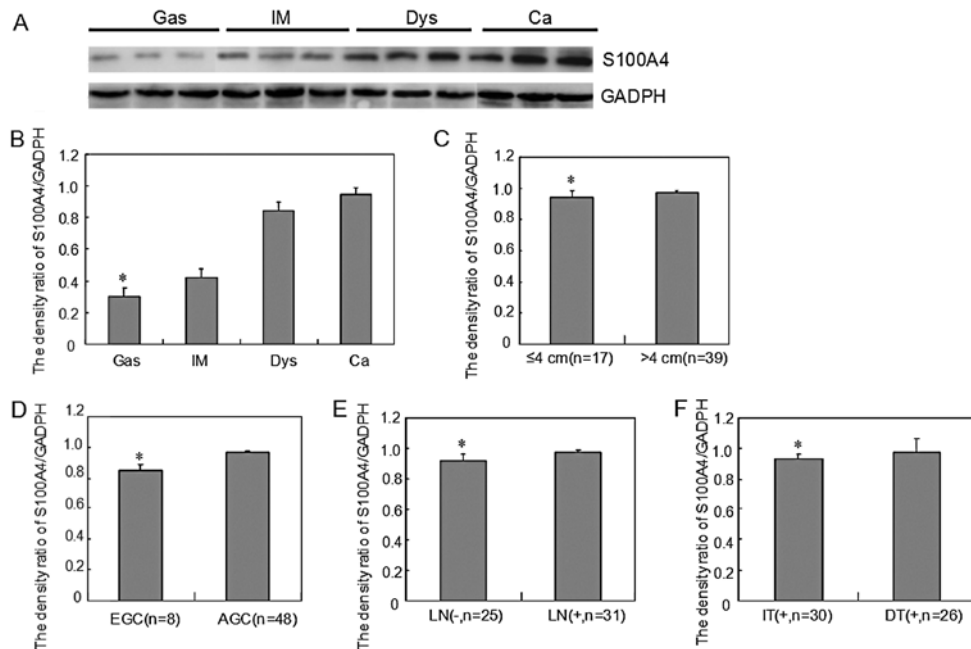

Figure 3. S100A4 protein expression level during gastric carcinogenesis and its correlation with clinicopathological features of carcinoma. (A) Cell lysates were separated on a 15% SDS-polyacrylamide gel, transferred to a Hybond membrane and probed with anti-S100A4 (29 kDa), using GADPH (37 kDa) as an internal control. (B) Densitometric analysis demonstrated S100A4 expression in gastritis (Gas), intestinal metaplasia (IM), dysplasia (Dys), and carcinoma (Ca) specimens, and the S1004A protein expression level gradually increased from Gas, IM, Dys to Ca (*P<0.05, Ca vs. Gas). S100A4 protein expression was higher in gastric carcinomas that showed (C) a larger diameter (*P<0.05, >4 cm vs. ≤4 cm), (D) deeper invasion (*P<0.05, AGC vs. EGC), (E) frequent lymph node metastasis [*P<0.05, LN(+) vs ≤LN(-)], and (F) intestinal-type (*P<0.05, DT vs. IT). Data are presented as means ± SD. EGC, early gastric cancer; AGC, advanced gastric cancer; LN, lymph node metastasis; IT, intestinal-type; DT, diffuse-type.

Real-time RT-PCR and western blot analysis for S100A4 expression and its correlation with clinicopathological features of gastric carcinoma. We designed S100A4-specific primers and performed real-time RT-PCR to quantify the mRNA expression level of S100A4 by using the house-keeping gene GAPDH as an internal control. The S100A4 mRNA expression was upregulated in the following order: gastritis < IM < dysplasia < carcinoma (P<0.001) (Fig. 2). Higher S100A4 mRNA expression was observed in gastric carcinomas that showed a larger diameter (>4 cm), deeper invasion, and frequent lymph node metastasis, and in IT carcinoma (P<0.05). These findings were also supported by the evaluation of S100A4 protein expression as assessed by western blot analysis (Fig. 3).

Table III. Multivariate analysis of clinicopathological variables to determine the survival of gastric carcinoma cases.

Clinicopathological parameters	Relative risk (95% CI)	P-value
Age (≥55 years)	1.399 (0.992-1.898)	0.057
Gender (female)	0.729 (0.519-1.241)	0.087
Depth of invasion (T2-4)	2.288 (1.689-6.234)	0.048
Lymphatic invasion (+)	1.528 (1.055-2.367)	0.049
Venous invasion (+)	1.672 (1.063-2.723)	0.041
Lymph node metastasis (+)	2.042 (1.114-3.931)	0.027
TNM staging (III-IV)	5.584 (2.126-12.045)	<0.001
Lauren's classification (intestinal/diffuse)	1.119 (0.886-1.393)	0.287
S100A4 expression (+ - +++)	1.522 (1.325-4.236)	0.045

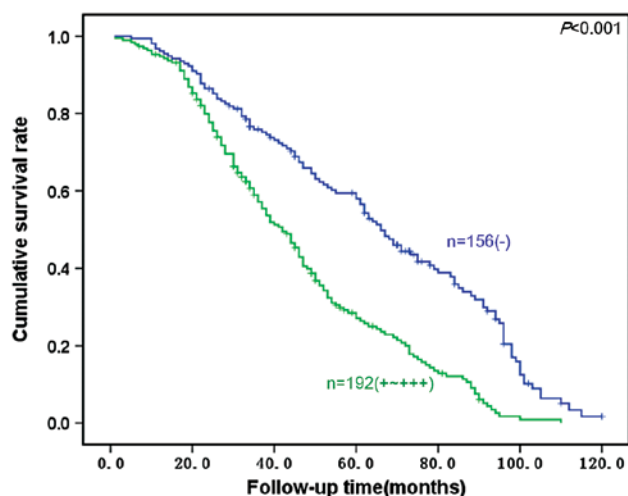CI, confidence interval; TNM, tumor-node-metastasis.

Figure 4. Correlation between S100A4 expression status and prognosis of gastric carcinoma patients. Kaplan-Meier curves were plotted to determine cumulative survival rate of patients with gastric carcinomas based on S100A4 expression. The cumulative survival rate of patients with weak or moderate S100A4 expression (green curve) was lower than that of patients not expressing S100A4 (blue curve; P<0.001).

Correlation between S100A4 expression and survival of gastric carcinoma patients. Follow-up information was available on 348 gastric carcinoma patients for periods ranging from 1 month to 10.1 years (median, 65.1 months). Fig. 4 shows survival curves stratified according to S100A4 protein expression in the gastric carcinomas. Univariate analysis using the Kaplan-Meier method indicated the cumulative survival rate of patients with weak or moderate S100A4 expression (n=192) to be clearly lower than that of patients not expressing S100A4 (n=156; P<0.05).

Multivariate analysis using Cox's proportional hazard model was conducted to determine the independent prognostic effects of the various clinicopathological parameters. Our results demonstrated that depth of invasion (P=0.048), lymphatic and venous invasion (P=0.049 and 0.041, respectively), lymph node metastasis (P=0.027), TNM staging (P<0.001), and S100A4 expression (P=0.045) were indepen-

dent factors for the poor prognosis of gastric carcinoma (all P<0.05) (Table III).

Discussion

In the present study, the S100A4 protein was detected in the cytoplasm and nuclei of gastric lesions, which is in agreement with a previous report (24,25). S100A4 is a transcriptional factor that can translocate from the nucleus to the cytosol under certain physiological or pathological conditions. Here, we found gradually increased expression of S100A4 at both the mRNA and protein level in the following ascending order: gastritis < IM < dysplasia < carcinoma. These results are in line with other reports regarding clear cell renal cell carcinoma (26), esophageal squamous cell carcinoma (ESCC) (27,28), colorectal carcinoma (29), breast cancer (30) and bladder cancer (31). IM is believed to be an adaptive condition for an injured and inflamed gastric epithelium and can develop into globoid dysplasia, which is closely linked to signet ring cell carcinoma, as revealed by its morphological appearance and biological characteristics (32). Pathological and genetic observations demonstrated that gastric dysplasia precedes the majority of carcinomas, can undergo malignant transformation, and is classified into cryptal, globoid, regenerative, and adenomatous subtypes (33). Our study is the first to suggest that higher S100A4 expression may contribute to carcinogenesis. Based on previous literature reports (34,35), S100A4 overexpression may result in hypomethylation of the CpG sites in the first intron of the S100A4 gene or simply a result of S100A4 stimulation under a hypoxic microenvironment.

To elucidate the role of the S100A4 protein in the progression of gastric carcinoma, we tested whether the expression status of S100A4 was associated with the aggressive behavior of carcinomas. We found that the S100A4 mRNA and protein expression was positively linked to tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis, and TNM staging in gastric carcinomas, which supported the previous findings of Feng *et al* (36). It has been reported that cytoplasmic expression of the S100A4 protein was positively correlated with depth of invasion and lymph node metastasis, whereas the nuclear expression of S100A4 protein was positively

correlated with the depth of invasion and perineural invasion of colorectal cancer (37). The positive relationship between S100A4 expression and aggressiveness was also observed in advanced-stage endometrial cancer (38) and bladder cancer (31). These findings suggest that S100A4 overexpression is involved in the growth, invasion, and metastasis of gastric carcinoma and may serve as an indicator of the aggressiveness of gastric carcinoma tumors in clinicopathological practice. In the light of our present findings, we speculate that S100A4 upregulation may participate in the progression of gastric cancer by reversing the aggressive phenotype of cancer cells.

S100A4 is known to regulate migratory and invasive behavior of human ESCC cells through the downregulation by E-cadherin expression and modulation of the AKT/Slug pathway (28,39). Sack et al (40) reported that S100A4-induced cell motility and metastasis are inhibited by the Wnt/β-catenin pathway inhibitor calcimycin in colon cancer cells. Zhang et al (27) reported that S100A4 mediates the cell invasion and metastasis of ESCC via the regulation of MMP-2 and E-cadherin activity. Shen et al (41) found that S100A4 protects gastric cancer cells from anoikis through regulation of αv and α5 integrins. Hua et al (42) demonstrated that inhibition of S100A4 promotes apoptosis and suppresses proliferation of BGC823 gastric cancer cells in vitro and in vivo. Xie et al (43) documented that S100A4 mediates endometrial cancer invasion, and induction of S100A4 was associated with the activation of Smads in tumor growth factor (TGF)-β1 signaling. Based on these data and our current findings, we hypothesize that S100A4 upregulation enhances the aggressive phenotypes and behavior of malignant cancers through the Wnt/β-catenin or TGF-β signaling pathways.

Although gastric cancer is a malignant tumor originating from the same type of gastric epithelium, its morphological features vary substantially among individual patients. According to Lauren's classification, IT carcinomas are characterized by cohesive carcinoma cells forming gland-like tubular structures with expanding or infiltrative growth patterns, and include well- and moderately differentiated adenocarcinomas. DT carcinomas display less apparent or no cell adhesion and contain poorly differentiated signet-ring cell carcinoma (22,23). In the present study, we noted that the protein and mRNA expression of S100A4 was lower in IT carcinoma compared to that in DT, indicating that S100A4 may play an important role in IT carcinogenesis, but less so in the de novo carcinogenic pathway, and may serve as the molecular basis for distinction between the two types of carcinomas.

In the present study, we found that S100A4 expression was inversely correlated with the favorable prognosis of patients with gastric carcinoma, which was consistent with previous reports (24,25). Cox's proportional risk analysis indicated that the depth of invasion, lymphatic or venous invasion, lymph node metastasis, TNM staging, and S100A4 expression were independent factors for the poor prognosis of gastric carcinoma patients. These findings suggest that S100A4 expression is an independent prognostic factor for gastric carcinoma patients. Kho et al (44) found that only cytoplasmic S100A4 staining, but not nuclear staining, of the frontal tissue was associated with poor survival of patients with stage C colonic cancer. In contrast, Kang et al (37) showed that nuclear expression of the S100A4 protein was found to be an independent prognostic

factor for poor survival of colorectal cancer patients. Similar results were also obtained in pancreatic cancer (45), renal cell carcinoma (46), bladder cancer (31) and lung squamous cell carcinoma (47). These findings suggest that S100A4 expression is an independent and reliable indicator for a less favorable prognosis of gastric cancer patients.

In summary, upregulation of S100A4 may play an important role in the malignant transformation of gastric epithelial cells and is positively related to growth, invasion, metastasis, and prognosis of gastric carcinomas. Thus, S100A4 may be considered as a promising marker to indicate the aggressive behavior of gastric carcinomas. The distinct expression of S100A4 in DT gastric carcinoma can be employed to differentiate between the intestinal- and diffuse-type carcinomas and may serve as a molecular feature that distinguishes the two types of carcinomas.

Acknowledgements

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