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STRA6 Expression Serves as a Prognostic Biomarker of Gastric Cancer

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Abstract. *Background: Despite advances in our understanding on the pathogenesis of gastric cancer (GC), patients face a poor prognosis. To improve clinical outcomes, effective approaches to diagnosis and treatment employing new diagnostic biomarkers are required to achieve early detection and predict recurrence and prognosis. Materials and Methods: Transcriptome analysis was conducted using surgically resected gastric tissues from four patients with metastatic GC. A total of 228 pairs of primary GC tissues and corresponding normal adjacent tissues were subjected to mRNA expression analysis. To validate our findings, we accessed an integrated microarray dataset and RNA sequencing data of GC cell lines. Results: We identified stimulated by retinoic acid 6 (STRA6) as a differentially overexpressed gene, which encodes a transmembrane protein that mediates the cellular uptake of retinol. To investigate how STRA6 contributes to the malignant phenotype of GC cells, we mined public datasets and found the mRNA encoding retinol binding protein 1 (RBP1), which is associated with retinoid metabolism, was co-expressed with STRA6. Furthermore, STRA6 mRNA levels were significantly higher in GC tissues compared to the corresponding noncancerous adjacent tissues of 228 surgically resected gastric tissue samples. Moreover, patients with high levels of STRA6 mRNA experienced significantly shorter disease-free survival and overall survival. Multivariate analysis revealed that high levels of STRA6 served as a significant risk factor. Conclusion: Patients with high levels of STRA6 mRNA experienced significantly worse clinical outcomes, indicating that STRA6 may serve as a diagnostic and prognostic biomarker of GC.*

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Gastric cancer (GC) is characterized by diverse genomic alterations. The molecular classification of GC described in The Cancer Genome Atlas as well as emerging molecular targeted and immune checkpoint therapy indicate the possibility for developing a genomics-based personalized strategy for treating patients with advanced disease (1-3). Despite advances in our understanding of the pathogenesis of GC, prognosis is poor, mainly due to early asymptomatic lymph node metastasis, leading to incurable hematogenous recurrence (4, 5). Further, peritoneal metastasis is difficult to detect when GC recurs (6-8). Therefore, effective diagnostic biomarkers for early detection and prediction of recurrence, as well as for disease prognosis are required (3, 9, 10).

For this purpose, we herein conducted a transcriptome analysis to identify genes expressed in association with the aggressive phenotype of GC. We focused on stimulated by retinoic acid 6 (*STRA6*), which was differentially overexpressed in primary GC tissue of patients with metastatic disease, because retinoid metabolism is considered to be involved in carcinogenesis. Previous studies have shown that *STRA6* expression is up-regulated in colorectal cancer and ovarian cancer (11, 12) although whether it is associated with GC is unknown. We, therefore, aimed to evaluate the clinical significance of *STRA6* expression in GC and explored its potential utility for diagnosis and predicting prognosis.

Materials and Methods

Participants informed consent. This study conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects and was approved by the Institutional Review Board of Nagoya

University, Japan (Study Approval Number 2014-0043). Written informed consent for use of clinical samples and data was obtained from all patients.

Transcriptome analysis. Surgically resected gastric tissues from four patients with metastatic GC were subjected to transcriptome analysis. Global expression profiling was conducted using the HiSeq platform (Illumina, San Diego, CA, USA) to compare the expression levels of 57,749 unique mRNAs expressed in primary GC tissues with those of the corresponding noncancerous adjacent gastric mucosa.

Reverse-transcription polymerase chain reaction (RT-PCR) analysis. The levels of *STRA6* mRNA in clinical samples were determined in triplicate using RT-PCR analysis as previously described (13, 14). The STRA6 primers were as follows: forward 5'-CTATGGCAGCTGGTACATCG-3' and reverse 5'-TACAG GCCGGGTGGTATG-3'. The level of the mRNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a standard for comparisons. Triplicate samples were subjected to quantitative real-time RT-PCR (qRT-PCR). The expression level of each sample is presented as the amount of the *STRA6* amplicon divided by that of *GAPDH*.

Clinical samples. From 2001 through 2017, 228 pairs of primary GC tissues and corresponding normal adjacent tissues were collected from surgical specimens of patients who underwent curative gastric resection for GC without neoadjuvant therapy at the Department of gastroenterological surgery, Nagoya University Hospital. Tissue samples were immediately frozen in liquid nitrogen after collection and stored at –80˚C. Specimens were histologically classified using the Union for International Cancer Control (UICC) classification, seventh edition. Patients were pathologically diagnosed with stages I–III GC, and relevant clinicopathological parameters were acquired from medical records. Since 2006, adjuvant chemotherapy using S-1 (an oral fluorinated pyrimidine) has been administered to all patients with UICC stage II-III gastric cancer, unless contraindicated by the patient's condition (15-17).

Bioinformatic analysis. To validate our experimental data, we accessed an integrated microarray dataset comprising the data of 1,065 patients from three major cancer Research Centers (Berlin, Bethesda, and Melbourne datasets; http://kmplot.com/analysis/). RNA sequencing data of 37 GC cell lines were obtained from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle). We used these datasets to determine the prognostic significance of *STRA6* mRNA expression and identify genes that were coordinately expressed with *STRA6*.

Statistical analysis. Qualitative variables were compared using the χ^2 test, and quantitative variables were compared using the Mann–Whitney test. Disease-free (DFS) and overall survival (OS) rates were calculated using the Kaplan–Meier method, and the difference in survival was analyzed using the log-rank test. We performed multivariate regression analysis with the Cox proportional hazards model to detect prognostic factors, and variables with $p<0.05$ were entered into the final model. To adjust for multiple testing, we employed a false discovery rate (FDR) approach and computed the q-value. Statistical analysis was performed using the JMP 14 software (SAS Institute, Cary, NC, USA) and R version 3.4.1 (Vienna, Austria. URL: http://www.R-project.org/). $p<0.05$ indicates a statistically significant difference.

Results

Identification of STRA6 as a putative driver of GC. We analyzed the transcriptomes of primary gastric cancer tissues of patients with metastatic GC to identify putative driver genes associated with an aggressive phenotype. We found that 31 genes were differentially expressed at higher levels in GC tissues compared to those of adjacent normal tissues (Table I). We focused on *STRA6* because of the association of retinol metabolites with the progression of GC and, to our knowledge, the absence data for *STRA6* expression in GC.

Correlation analysis of STRA6 expression using an external dataset. To evaluate the role of *STRA6* in cancer progression, we performed correlation analysis using the CCLE dataset to identify genes differentially expressed in association with *STRA6*. The top five mRNAs with the highest correlation, according to the adjusted false discovery rate (FDR), are listed in Table II. We focused on the gene encoding retinol-binding protein 1 (*RBP1*), which serves as a carrier protein involved in the transport of retinol. These data suggest that STRA6 and RBP1, which are related to retinol metabolism, may activate a common signaling pathway leading to gastric carcinogenesis.

Clinical significance of STRA6 expression in surgically resected gastric tissue. The median age of the 228 patients was 67 years (range=26-96 years), and the male:female ratio was 164:64. According to the UICC staging system (seventh edition), 50, 71, and 107 patients exhibited the characteristics of GC pathological stages I, II, and III, respectively. *STRA6* mRNA levels were significantly higher in GC tissues compared to those of the corresponding noncancerous adjacent tissues. Moreover, higher levels of *STRA6* mRNA were expressed during the late stages of GC (Figure 1A). Patients were assigned according to their median *STRA6* mRNA level in GC tissues as follows: high-STRA6 expression group, n=114; low-STRA6 expression group, n =114. The characteristics of two groups are presented in Table III. Most key clinical variables, except the UICC stage, were equally distributed between groups. Higher levels of *STRA6* mRNA were significantly associated with shorter DFS and OS [hazard ratio (HR)=2.27, 95% confidence interval (CI)=1.29-3.98, $p=0.004$; HR=3.14, 95% CI=1.52-6.45, $p=0.002$, respectively] (Figure 1B). Multivariate analysis revealed that high levels of *STRA6* mRNA had the highest HR among candidate risk factors (HR=2.42, 95% CI=1.14–5.13, $p=0.021$) (Table IV).

To further investigate the clinical significance of STRA6, we assessed survival rates using the prognostic data acquired

Table I. *List of candidate genes expressed at increased levels in primary tumor tissues of patients with metastatic gastric cancer.*

Biological function	Symbol	Full name	Location	Log_2 ratio	*p*-Value
Metabolic enzyme	*PLA2G2A*	Phospholipase A2 group IIA	1p36.13	3.7	<0.0001
	STRA6	Stimulated by retinoic acid 6	15q24.1	3.41	<0.0001
	AKR1C4	Aldo-keto reductase family 1 member C4	10p15.1	3.28	0.0009
	KLK10	Kallikrein related peptidase 10	19q13.41	3.26	0.0003
	PADI2	Peptidyl arginine deiminase 2	1p36.13	3.01	<0.0001
	FKBP10	FKBP prolyl isomerase 10	17q21.2	2.77	<0.0001
Transcription factor	*HOXC10*	Homeobox C10	12q13.13	6.49	0.0001
	ELF5	E74 like ETS transcription factor 5	11p13	5	0.0001
	BEX1	Brain expressed X-linked 1	Xq22.1	3.46	<0.0001
TGF-beta superfamily	*INHBA*	Inhibin beta A subunit	7p14.1	3.76	<0.0001
	INHBB	Inhibin subunit beta B	2q14.2	3.46	<0.0001
Regulator of cell cycle	*CCNE1*	Cyclin E1	19q12	3.41	<0.0001
	CDC25B	Cell division cycle 25B	20p13	3.17	0.0006
Keratin family	*KRT6B*	Keratin 6B	12q13.13	6.83	0.0006
	KRT80	Keratin 80	12q13.13	4.29	0.0001
Cell adhesive glycoprotein	*THBS4*	Thrombospondin 4	5q14.1	4.01	<0.0001
	THBS2	Thrombospondin 2	6q27	3.76	<0.0001
Activator of G protein signaling	*FNDC1*	Fibronectin type III domain containing 1	6q25.3	4.5	<0.0001
Adapter of tyrosine kinase receptors	*GRB7*	Growth factor receptor bound protein 7	17q12	3.98	<0.0001
Cancer antigen	*PRAME*	Preferentially expressed antigen in melanoma	22q11.22	4.79	0.0054
Central nervous neuropeptide	*NPY*	Neuropeptide Y	7p15.3	4.86	<0.0001
Component of tight junction strands	*CLDN1*	Claudin 1	3q28	3.27	<0.0001
Extracellular matrix protein	*COMP*	Cartilage oligomeric matrix protein	19p13.11	3.15	0.0003
Membrane trafficking protein	*SYT7*	Synaptotagmin 7	11q12.2	4.29	<0.0001
Neuronal calcium sensor protein	*VSNL1*	Visinin like 1	2p24.2	4.04	<0.0001
Protein folding and export	*DNAJC12*	DnaJ heat shock protein family member C12	10q21.3	4.15	<0.0001
Receptor of G-proteins	*UTS2R*	Urotensin 2 receptor	17q25.3	4.5	<0.0001
Signal transducer	*GNG4*	G protein subunit gamma 4	1q42.3	4.84	<0.0001
Specific carrier for retinol	*RBP4*	Retinol binding protein 4	10q23.33	4.25	<0.0001
Synaptic vesicle exocytosis	*CPLX2*	Complexin 2	5q35.2	4.36	0.0007
TNF-receptor	*TNFRSF11B*	TNF receptor superfamily member 11b	8q24.12	4.57	<0.0001

Table II. *List of genes strongly correlated with STRA6 mRNA expression level in GC cell lines.*

Symbol	Location	Full name	Biological function	r	FDR q value
VWA2	10q25.3	von Willebrand factor A domain containing 2	von Willebrand factor A domain-containing protein	0.83	<0.0001
RBP1	3q23	Retinol binding protein 1	Retinol uptake	0.8	<0.0001
NKD1	16q12.1	Naked cuticle homolog 1	Negative modulator of the canonical Wnt/β catenin pathway	0.8	<0.0001
NAPG	18p11.22	NSF attachment protein gamma	Synaptic transmission	0.79	<0.0001
OR2J2	6p22.1	Olfactory receptor family 2 subfamily J member 2	G-protein-coupled receptors	0.78	<0.0001
DEFA5	8p23.1	Defensin alpha 5	Antimicrobial and cytotoxic peptides	0.76	<0.0001

FDR, False discovery rate.

from external validation datasets. A consistent result was observed in the extra-validation cohort of 444 patients with stage I-III GC (Figure 1C). Furthermore, the frequencies of initial recurrence sites, particularly lymph node metastasis, were significantly higher in the high-STRA6 expression group (Figure 2).

Discussion

Herein we identified *STRA6* as a candidate causative gene of GC that was associated with highly malignant tumor cell phenotypes. Moreover, patients with relatively higher levels of *STRA6* mRNA in GC tissues *vs*. controls experienced

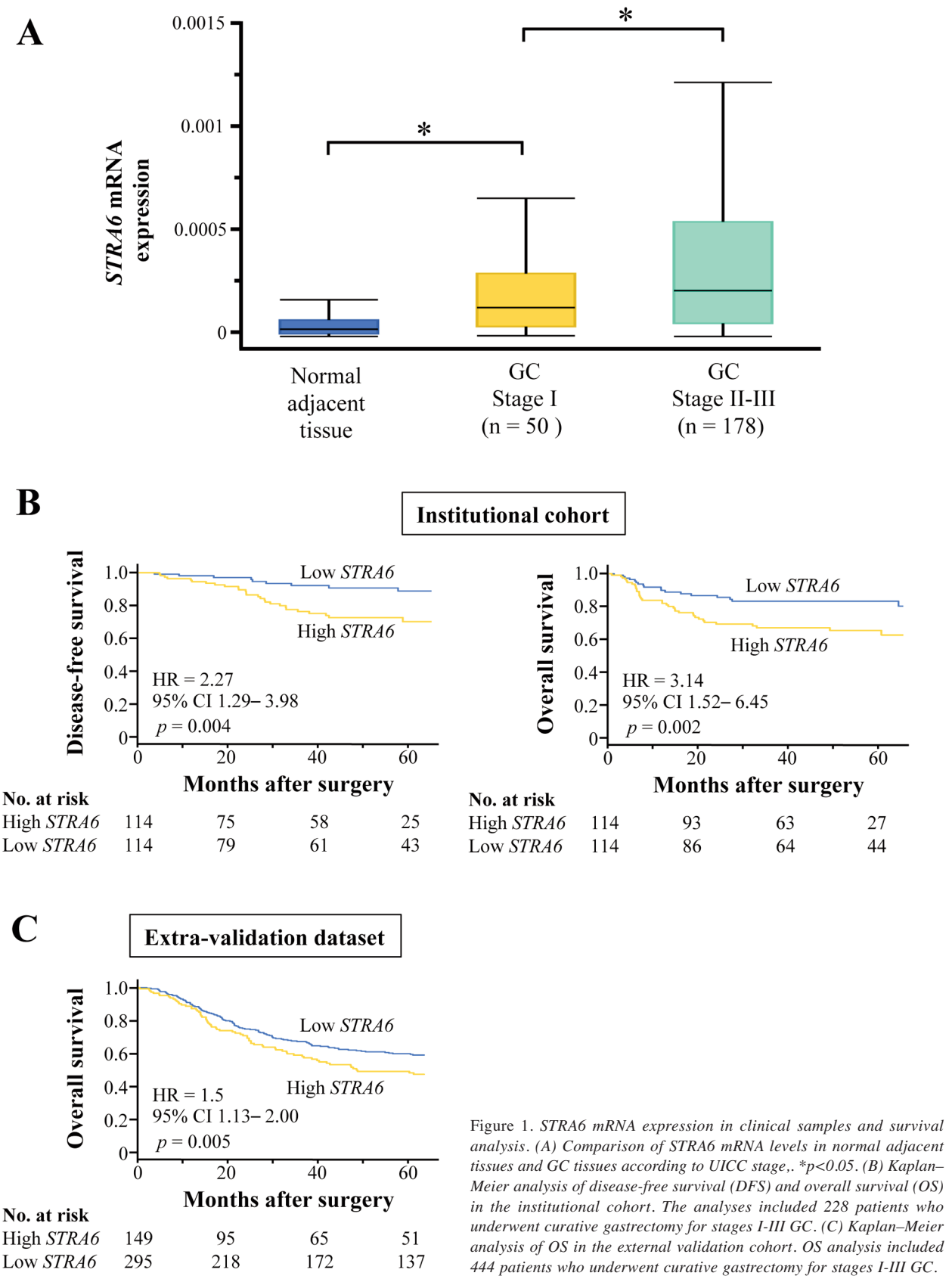

Figure 1. *STRA6 mRNA expression in clinical samples and survival analysis. (A) Comparison of STRA6 mRNA levels in normal adjacent tissues and GC tissues according to UICC stage,. *p<0.05. (B) Kaplan–Meier analysis of disease-free survival (DFS) and overall survival (OS) in the institutional cohort. The analyses included 228 patients who underwent curative gastrectomy for stages I-III GC. (C) Kaplan–Meier analysis of OS in the external validation cohort. OS analysis included 444 patients who underwent curative gastrectomy for stages I-III GC.*

Table III. *Association between the expression of STRA6 mRNA and clinicopathological parameters of 228 patients with stage I-III gastric cancer.*

Parameters	Low *STRA6* expression (n)	High *STRA6* expression (n)	*p*-Value
Age (year)			
<65	45	53	0.3491
≥65	69	61	
Gender			
Male	79	85	0.4613
Female	35	29	
Body mass index			
<25	93	91	0.8669
≥25	21	23	
CEA (ng/ml)			
≤5	98	96	1.0000
>5	16	16	
CA19-9 (ng/ml)			
≤37	98	87	0.2177
>37	16	23	
Macroscopic type			
Type 4/5	10	11	1.0000
Others	104	103	
Tumor size (cm)			
<6.0	78	81	0.7732
≥6.0	36	33	
Tumor location			
Lower	47	33	0.0709
Others	67	81	
UICC pT factor			
pT1-3	78	69	0.2682
T4	36	45	
Differentiation			
Poor	62	67	0.5931
Others	52	47	
Lymphatic involvement			
Absent	23	14	0.1501
Present	91	100	
Vessel invasion			
Absent	46	41	0.5857
Present	68	73	
Infiltrative growth			
a/b	81	77	0.6668
c	33	37	
Lymph node metastasis			
Absent	51	36	0.0560
Present	63	78	
UICC stage			
I	33	17	0.0158
II/III	81	97	

χ^2 test. *Statistically significant ($p<0.05$, two-tailed). BMI CEA, Carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; UICC, Union for International Cancer Control.

significantly shorter OS. To the best of our knowledge, the present report is the first to implicate *STRA6* as a putative oncogene in GC.

STRA6 serves as a cytokine signaling receptor, in addition to its role as a retinol transporter, and activates a JAK2/STAT3 cascade through retinol uptake and intracellular metabolism, leading to induction of multiple pro-oncogenic STAT target genes (18-20). Karunanithi *et al*. (12) showed that this STRA6/JAK2/STAT3 signaling cascade is one putative mechanism underlying the tumorigenesis of colon cancer. To investigate the interactions between STRA6 and other proteins that contribute to the malignant phenotype of GC cells, we investigated *STRA6* expression using the CCLE datasets, and found that the expression of *RBP1* significantly associated with that of *STRA6* in GC cell lines. RBP1, which is the intracellular acceptor of retinol, triggers phosphorylation of a tyrosine residue in the cytosolic domain of STRA6, resulting in recruitment and activation of the JAK2 and, in a cell-dependent manner, the transcription factor STAT3 (18, 19). Consequently, RBP1 contributes to activation of the STRA6/JAK2/STAT3 signaling cascade, and triggers inflammation, proliferation, invasion, and angiogenesis. These findings further support the conclusion that *STRA6* may participate in GC progression through the JAK2/STAT3 signaling cascade.

We next evaluated the association of *STRA6* mRNA levels in GC tissues with patient clinical characteristics. Most clinical variables were equally distributed between the two *STRA6*-expression groups, except UICC stage. We found that the levels of *STRA6* mRNA in GC tissues were significantly higher compared to those of the paired noncancerous tissues. Moreover, the levels of *STRA6* mRNA were higher in the cancerous tissues of patients with the later stages of disease. These findings support the conclusion that elevated *STRA6* expression may contribute to the initiation and progression of GC.

We observed significant differences between OS and DFS between the two *STRA6* expression groups, and patients in the high *STRA6* expression group experienced worse outcomes. These clinical findings are consistent with the OS of patients in the external validation dataset. Moreover, multivariate analysis revealed that high levels of *STRA6* mRNA served as an independent risk factor of shorter OS. Further, higher levels of *STRA6* predicted a higher incidence of recurrence, particularly lymph node metastasis. Considering that the number of patients with metastatic lymph nodes was larger (although the difference was not statistically different), the finding of a higher incidence of lymph node recurrence in the high-*STRA6* group was unsurprising. Together, these results support the conclusion that *STRA6* mRNA levels will serve as a biomarker for early detection as well as prediction of prognosis and detection of recurrence of GC

Carcinoembryonic antigen and carbohydrate antigen 19-9 are widely employed as biomarkers of GC, (21, 22) although our multivariate analysis revealed that elevated levels of these biomarkers were not significantly associated with patient survival. Moreover, the present study indicates that

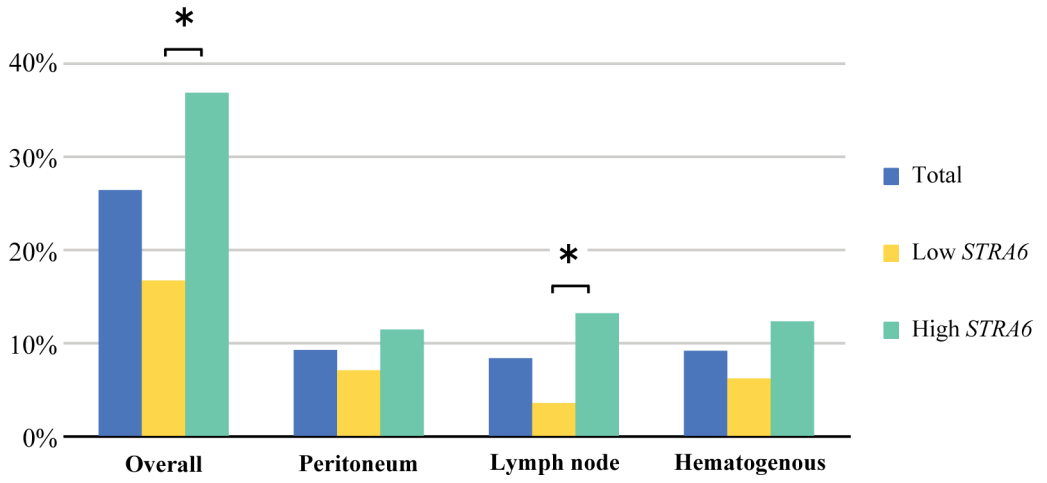

Figure 2. *Frequencies of initial recurrence after curative gastrectomy in patients according to STRA6 mRNA levels. *p<0.05.*

Table IV. *Prognostic factors for overall survival of patients with stage I-III gastric cancer (n=228).*

Variable	n	Univariate			Multivariable		
		Hazard ratio	95% CI	*p*-Value	Hazard ratio	95% CI	*p*-Value
Age (≥65 year)	130	0.98	0.52-1.85	0.949	1.20	0.61-2.39	0.600
Gender (male)	164	0.94	0.47-1.89	0.862	1.26	0.59-2.69	0.553
Body mass index (≥25)	44	0.85	0.37-1.93	0.697			
CEA (≥ 5 ng/ml)	32	1.43	0.63-3.25	0.394			
CA19-9 (≥37 ng/ml)	39	2.61	1.28-5.32	0.008	1.64	0.77-3.48	0.199
Macroscopic type (4/5)	21	1.90	0.79-4.54	0.148			
Tumor size (≥6.0 cm)	69	2.64	1.41-4.95	0.003	2.09	1.07-4.08	**0.031**
Tumor location (lower)	80	0.73	0.37-1.44	0.364			
UICC T factor (T4)	81	3.44	1.80-6.57	<0.001	1.68	0.71-3.98	0.242
Differentiation (poor)	129	1.73	0.89-3.37	0.107			
Lymphatic involvement (present)	191	5.08	1.22-21.18	0.026	0.42	0.07-2.45	0.335
Vessel invasion (present)	141	3.48	1.59-7.61	0.002	2.25	0.91-5.55	0.077
Infiltrative growth (c)	70	2.12	1.12-4.02	0.021	1.64	0.77-3.49	0.195
Lymph node metastasis (present)	141	11.46	3.50-37.52	<0.001	3.78	0.77-18.68	0.103
UICC stage (stage III)	108	7.08	3.12-16.08	<0.001			
Adjuvant chemotherapy (performed)	106	1.37	0.73-2.58	0.323	2.36	0.70-7.97	0.168
STRA6 (high)	114	3.13	1.52-6.45	0.002	2.42	1.14-5.13	**0.021**

CEA, Carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; UICC, Union for International Cancer Control; *STRA6*, stimulated by retinoic acid 6. Bold values indicate statistical significance.

the assessment of *STRA6* mRNA levels in surgically resected GC tissue, or biopsy specimens obtained from preoperative endoscopic investigations, may provide prognostic information about patients with GC. We, therefore, recommend that patients with high levels of *STRA6* mRNA should be considered as candidates for undergoing enhanced perioperative chemotherapy.

There are several limitations to the present study. First, we analyzed data acquired from a small number of patients treated at a single Institution. However, we found that our analysis of an external validation dataset was consistent with that of our patients. Further, the cut-off value of *STRA6* levels and the sensitivity and specificity of detection require further optimization. Second, information about chemotherapy was unavailable, because the study's subjects were acquired over a long period during which the chemotherapeutic regimen was progressively updated. It is important to note that neoadjuvant chemotherapy is not a standard treatment for GC in Japan. Third, direct functional analyses of STRA6 were not conducted herein. Molecular genetics techniques such as

RNA interference, transgenic expression of *STRA6*, and CRISPR/Cas9 mutagenesis will likely lead to a better understanding of the underlying mechanism of the effects of STRA6 on the pathobiology of GC. Our study provides compelling evidence that the JAK2/STAT3 cascade contributes to *STRA6*-mediated tumor progression. Nevertheless, further research is required to decipher the signal transduction pathways that mediate the putative oncogenic effects of S*TRA6* on gastric epithelial cells.

In conclusion, patients with high levels of *STRA6* mRNA experienced significantly worse clinical outcomes, indicating that *STRA6* may serve as diagnostic and prognostic biomarker of GC.

Conflicts of Interest

The Authors have no conflicts of interest with regard to the present study.

Authors' Contributions

SN and MK conceived the study concept and design, analysed data and wrote the manuscript. MK, DS, MK and YK contributed to data acquisition and interpretation. KO contributed to statistical analysis. CT, NH, MH, SY, GN and YK revised the draft. All Authors have read and approved the final version of the manuscript.

References

1 Figueiredo C, Camargo MC, Leite M, Fuentes-Panana EM, Rabkin CS and Machado JC: Pathogenesis of gastric cancer: Genetics and molecular classification. Curr Top Microbiol Immunol *400*: 277-304, 2017. PMID: 28124158. DOI: 10.1007/978-3-319-50520-6_12

2 Katona BW and Rustgi AK: Gastric cancer genomics: advances and future directions. Cell Mol Gastroenterol Hepatol *3(2)*: 211-217, 2017. PMID: 28275688. DOI: 10.1016/j.jcmgh.2017.01.003

3 Nakamura S, Kanda M and Kodera Y: Incorporating molecular biomarkers into clinical practice for gastric cancer. Expert Rev Anticancer Ther *19(9)*: 757-771, 2019. PMID: 31437076. DOI: 10.1080/14737140.2019.1659136

4 Umeda S, Kanda M, Miwa T, Tanaka H, Tanaka C, Kobayashi D, Hayashi M, Yamada S, Nakayama G, Koike M and Kodera Y: Fraser extracellular matrix complex subunit 1 promotes liver metastasis of gastric cancer. Int J Cancer *146*: 2865-2876, 2019. PMID: 31597194. DOI: 10.1002/ijc.32705

5 Kanda M, Tanaka H, Shimizu D, Miwa T, Umeda S, Tanaka C, Kobayashi D, Hattori N, Suenaga M, Hayashi M, Iwata N, Yamada S, Fujiwara M and Kodera Y: SYT7 acts as a driver of hepatic metastasis formation of gastric cancer cells. Oncogene *37(39)*: 5355-5366, 2018. PMID: 29858600. DOI: 10.1038/s41388-018-0335-8

6 Kanda M, Shimizu D, Tanaka H, Tanaka C, Kobayashi D, Hayashi M, Takami H, Niwa Y, Yamada S, Fujii T, Sugimoto H and Kodera Y: Synaptotagmin XIII expression and peritoneal metastasis in gastric cancer. Br J Surg *105(10)*: 1349-1358, 2018. PMID: 29741294. DOI: 10.1002/bjs.10876

7 Nakanishi K, Kanda M, Umeda S, Tanaka C, Kobayashi D, Hayashi M, Yamada S and Kodera Y: The levels of SYT13 and CEA mRNAs in peritoneal lavages predict the peritoneal recurrence of gastric cancer. Gastric Cancer *22(6)*: 1143-1152, 2019. PMID: 31055693. DOI: 10.1007/s10120-019-00967-3

8 Kanda M, Shimizu D, Tanaka H, Tanaka C, Kobayashi D, Hayashi M, Iwata N, Niwa Y, Yamada S, Fujii T, Sugimoto H, Murotani K, Fujiwara M and Kodera Y: Significance of SYT8 for the detection, prediction, and treatment of peritoneal metastasis from gastric cancer. Ann Surg *267(3)*: 495-503, 2018. PMID: 28026832 DOI: 10.1097/SLA.0000000000002096

9 Kanda M, Suh YS, Park DJ, Tanaka C, Ahn SH, Kong SH, Lee HJ, Kobayashi D, Fujiwara M, Shimada H, Cho B, Murotani K, Kim HH, Yang HK and Kodera Y: Serum levels of ANOS1 serve as a diagnostic biomarker of gastric cancer: A prospective multicenter observational study. Gastric Cancer *23*: 203-211, 2019. PMID: 31377880. DOI: 10.1007/s10120-019-00995-z

10 Virgilio E, Proietti A, D'Urso R, Cardelli P, Giarnieri E, Giovagnoli MR, Montagnini M, Villani S, Balducci G and Cavallini M: Elevated gastric juice carbohydrate antigen 72.4 (Ca 72.4) is an independent prognostic factor of poor survival for gastric cancer patients. Anticancer Res *40(3)*: 1691-1695, 2020. PMID: 32132076. DOI: 10.21873/anticanres.14121

11 Szeto W, Jiang W, Tice DA, Rubinfeld B, Hollingshead PG, Fong SE, Dugger DL, Pham T, Yansura DG, Wong TA, Grimaldi JC, Corpuz RT, Singh JS, Frantz GD, Devaux B, Crowley CW, Schwall RH, Eberhard DA, Rastelli L, Polakis P and Pennica D: Overexpression of the retinoic acid-responsive gene Stra6 in human cancers and its synergistic induction by Wnt-1 and retinoic acid. Cancer Res *61(10)*: 4197-4205, 2001. PMID: 11358845.

12 Karunanithi S, Levi L, DeVecchio J, Karagkounis G, Reizes O, Lathia JD, Kalady MF and Noy N: RBP4-STRA6 pathway drives cancer stem cell maintenance and mediates high-fat diet-induced colon carcinogenesis. Stem Cell Reports *9(2)*: 438-450, 2017. PMID: 28689994. DOI: 10.1016/j.stemcr.2017.06.002

13 Uno Y, Kanda M, Miwa T, Umeda S, Tanaka H, Tanaka C, Kobayashi D, Suenaga M, Hattori N, Hayashi M, Yamada S, Nakayama G, Fujiwara M and Kodera Y: Increased expression of DNAJC12 is associated with aggressive phenotype of gastric cancer. Ann Surg Oncol *26(3)*: 836-844, 2019. PMID: 30617870. DOI: 10.1245/s10434-018-07149-y

14 Umeda S, Kanda M, Koike M, Tanaka H, Miwa T, Tanaka C, Kobayashi D, Suenaga M, Hayashi M, Yamada S, Nakayama G and Kodera Y: Copine 5 expression predicts prognosis following curative resection of esophageal squamous cell carcinoma. Oncol Rep *40(6)*: 3772-3780, 2018. PMID: 30272363. DOI: 10.3892/or.2018.6742

15 Japanese Gastric Cancer A: Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer *20(1)*: 1-19, 2017. PMID: 27342689. DOI: 10.1007/s10120-016-0622-4

16 Nakanishi K, Kanda M, Ito S, Mochizuki Y, Teramoto H, Ishigure K, Murai T, Asada T, Ishiyama A, Matsushita H, Tanaka C, Kobayashi D, Fujiwara M, Murotani K and Kodera Y: Delay in initiation of postoperative adjuvant chemotherapy with S-1 monotherapy and prognosis for gastric cancer patients: Analysis of a multi-institutional dataset. Gastric Cancer *22(6)*: 1215-1225, 2019. PMID: 30955110. DOI: 10.1007/s10120-019-00961-9

17 Kanda M, Murotani K, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M and Kodera

Y: Postoperative adjuvant chemotherapy with S-1 alters recurrence patterns and prognostic factors among patients with stage II/III gastric cancer: A propensity score matching analysis. Surgery *158(6)*: 1573-1580, 2015. PMID: 26120068. DOI: 10.1016/j.surg.2015.05.017

18 Noy N: Vitamin a transport and cell signaling by the retinol-binding protein receptor STRA6. Subcell Biochem *81:* 77-93, 2016. PMID: 27830501 DOI: 10.1007/978-94-024-0945-1_3

19 Berry DC, Levi L and Noy N: Holo-retinol-binding protein and its receptor STRA6 drive oncogenic transformation. Cancer Res *74(21)*: 6341-6351, 2014. PMID. 25237067. DOI: 10.1158/0008-5472.CAN-14-1052

20 Carrera S, Cuadrado-Castano S, Samuel J, Jones GD, Villar E, Lee SW and Macip S: Stra6, a retinoic acid-responsive gene, participates in p53-induced apoptosis after DNA damage. Cell Death Differ *20(7)*: 910-919, 2013. PMID: 23449393. DOI: 10.1038/cdd.2013.14

21 Kanda M, Murotani K, Tanaka H, Miwa T, Umeda S, Tanaka C, Kobayashi D, Hayashi M, Hattori N, Suenaga M, Yamada S, Nakayama G, Fujiwara M and Kodera Y: Integrated multigene expression panel to prognosticate patients with gastric cancer. Oncotarget *9(27)*: 18775-18785, 2018. PMID: 29721160. DOI: 10.18632/oncotarget.24661

22 Kanda M and Kodera Y: Recent advances in the molecular diagnostics of gastric cancer. World J Gastroenterol *21(34)*: 9838-9852, 2015. PMID: 26379391. DOI: 10.3748/wjg.v21.i34.9838

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