FAM46C Serves as a Predictor of Hepatic Recurrence in Patients with Resectable Gastric Cancer

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Synopsis

Patients with gastric cancer have been suffering from disease recurrences. We found that reduced expression of family with sequence similarity 46, member C is a promising biomarker to predict hepatic recurrence after curative gastrectomy.

ABSTRACT

Background. Gastric cancer (GC) relapse can occur even if curative resection is achieved. Biomarkers predicting recurrence are needed to provide appropriate postoperative surveillance and perioperative therapeutic strategy.

Methods. A global expression profiling was performed using tissues from GC patients with synchronous liver-confined metastasis. Family with sequence similarity 46, member C (*FAM46C*) was identified as a candidate biomarker. mRNA expression analysis, direct nucleotide sequencing, bisulfite sequencing and copy number assays for *FAM46C* were performed with eleven GC cell lines. Expression levels of *FAM46C* in primary GC tissues from 129 patients who underwent curative GC resection were determined and correlated with clinicopathological factors including postoperative outcome.

Results. Levels of *FAM46C* mRNA differed among GC cell lines. Point mutations in *FAM46C* were detected in five GC cell lines accompanied with reduced *FAM46C* transcription. No hypermethylation was found in the promoter region of *FAM46C*. Copy number alterations were found in six GC cell lines with differing *FAM46C* transcription levels. Reduced *FAM46C* mRNA expression levels were detected in 117 (91%) GC specimens compared with adjacent noncancerous tissues. Low *FAM46C* expression levels were significantly associated with larger macroscopic GC tumor sizes. The low *FAM46C* expression group was likely to have shorter disease-free survival than the high group and low *FAM46C* level was identified as an independent risk factor for recurrence

after curative resection. *FAM46C* expression levels were low in all cases that were later found to have hepatic recurrence.

Conclusions. Reduced GC expression of *FAM46C* is a potential biomarker to predict hepatic recurrence after curative gastrectomy.

Gastric cancer (GC) is one of the most common cancers and the third leading cause of cancer-related deaths in the world.[1] GC relapse can occur even if the standard treatment of R0 resection followed by postoperative adjuvant chemotherapy is performed.[2,3] Sensitive biomarkers capable of detecting micrometastasis and predicting recurrence are yet to be identified. The “classical” tumor markers for GC such as carcinoembryonic antigen or carbohydrate antigen (CA) 19-9, which have been widely used for over three decades, have limited sensitivity and specificity.[4,5]

Recently, global gene expression analyses using next generation sequencing and/or microarrays have been employed by researchers to determine the molecular characteristics of cancer with distant metastasis and to identify numerous candidate genes possibly related to the metastatic potential of cancer cells.[6-8] However, absence of detailed information of individual genes identified by these comprehensive analyses led to insufifcient understanding of their underlying roles and diagnostic values, and thus prevented their use in clinical applications.

Here, we conducted a transcriptome analysis to explore genes reflecting the metastatic potential of GC cells and identified family with sequence similarity 46, member C (*FAM46C*) as a candidate biomarker to predict recurrence. To validate the results of our transcriptome analysis, expression, regulatory mechanisms and clinical implications of *FAM46C* were evaluated. To the best of our knowledge, this is the first study that explored role of *FAM46C* in GC.

MATERIALS AND METHODS

Global Expression Profiling Analysis

Transcriptome profiling was performed on surgical-resected specimens of four GC patients with synchronous liver metastasis. Global expression profiling was conducted using the HiSeq platform (Illumina, San Diego, CA, USA) to compare expression levels of 57761 genes among primary GC tissues and noncancerous adjacent gastric mucosa.[9]

Sample Collection

Eleven GC cell lines were used in this study. These included MKN1, MKN45, MKN74, NUGC2, NUGC3, NUGC4 and SC-6-JCK obtained from the Japanese Collection of Research Bio Resources Cell Bank (JCRB, Osaka, Japan), AGS, KATOIII and N87 obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and GCIY obtained from Cell Resource Center for Biomedical Research/Cell Bank (Sendai, Japan). A control, non-tumorigenic epithelial cell line (FHs74) was purchased from ATCC.[10] This study included 129 patients who underwent curative gastrectomy for stage I to III GC according to the 7th edition of the Union for International Cancer Control (UICC 7th) at the Department of Gastroenterological Surgery, Nagoya University Hospital between 2001 and 2014.[11,12] Tumor samples from pretreated cancer patients and those that were of insufficient size, because they were from early-stage GC, were excluded. Primary GC tissues and noncancerous adjacent tissues were collected immediately after resection and frozen by liquid

nitrogen. Frozen tissues were divided into small pieces and stored at -80 °C until DNA and RNA extraction without thaw and freeze process. Relevant clinical data were retrieved from the prospectively compiled departmental database.

This study conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, and written informed consent for the use of clinical samples and data, as required by the Institutional Review Board at Nagoya University, Japan, was obtained from all patients.

Analysis of FAM46C mRNA Levels

RNeasy® Mini Kit (Qiagen, Hilden, Germany) was used for RNA extraction according to the manufacture's protocol. Total RNAs (10 μg) isolated from each of the GC cell lines and surgically-resected gastric tissues were used as templates to generate complementary DNAs (cDNAs) by Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen, Carlsbad, CA). A quality check for all RNA samples was conducted before generating complementary DNAs. The optical density was measured and the ratio of the absorbance at 260 and 280 nm ranged from 1.8 to 2.0 in all samples. *FAM46C* mRNA levels were determined using a quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) assay with an ABI Step One Plus Real-Time PCR System (Applied Biosystems, CA, USA), as described previously.[13] The expression level of each sample was calculated as the value of the *FAM46C* amplicon divided by that of GAPDH. Specific primers are

listed in Supplemental Table 1.

Methylation, Mutation and Copy Number Analysis of FAM46C gene

To determine the amount of methylation of the *FAM46C* promoter region, bisulfite sequencing of the GC cell lines was performed as described previously.[14] Primers for sequencing of bisulfite converted genomic DNA were designed targeting CpG islands at the promoter region of FAM46C gene (Supplemental Table 1). The nucleotide sequence of the coding exon (exon 2) of the *FAM46C* gene was analyzed to determine the presence or absence of mutations as follows: exons, divided into five regions, were amplified using PCR, followed by purification of the PCR products, as described previously, and sequencing using Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo fisher scientific, Waltham, MA, USA) and a 3730 x l DNA Analyzer (Applied Biosystems) at Eurofins Genomics Co Ltd, Tokyo, Japan. The copy number at *FAM46C* locus was determined using Taq Man Copy Number Assays (Applied Biosystems) in the cell lines and clinical samples, as described previously.[15] Two assays were employed as follows: upstream (assay ID: Hs02054906, within exon 1 of *FAM46C*) and downstream (assay ID: Hs02842058 within exon 2 of *FAM46C*). Data were analyzed using Copy Caller Software (Applied Biosystems).

Statistical Analysis

The significance of the difference between variables of two groups was assessed using the

Mann–Whitney test. Fisher's exact test was used to analyze categorical data of the two groups. Overall survival and recurrence-free survival rates were calculated using the Kaplan–Meier method, and the difference between survival rates was evaluated using the log-rank test. Risk factors for disease recurrence were assessed using the COX hazard ratio model. A *P* value <0.05 was considered statistically significant. All statistical analyses were performed using R software (The R Foundation for Statistical Computing, Vienna, Austria) on EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R. More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[16]

RESULTS

Identification of FAM46C as a Candidate Biomarker

Using global expression analysis, 21 candidate downregulated genes were identified (Table 1). Opting for genes that are known to function as translation regulators and taking into account novelty of each gene, we selected *FAM46C* for further analysis with following reasons. *FAM46C*, whose expression or role has not been previously reported in GC, has been shown to play a role in the regulation of translation and to have a mutation that is predictive of a worse prognosis in about 10% of multiple myeloma patients.[17] In addition, our pilot data showed that *FAM46C* mRNA expression levels were suppressed in cancerous tissues.

FAM46C mRNA Levels, Methylation, Mutation and Copy number Status in GC Cell Lines

Figure 1a shows that the level of *FAM46C* mRNA differed among GC cell lines. In MKN74, GCIY, NUGC2, NUGC3, NUGC4 and MKN45 cell lines, *FAM46C* mRNA levels were downregulated compared with the control cell line FHs74. Figure 1a also shows that there is a list of *FAM46C* mRNA expression levels with corresponding data from assays used to detect genetic and epigenetic alterations. Two point mutations were detected in MKN74, GCIY, NUGC2, NUGC3 and MKN45 cell lines (Fig. 1b), and interestingly, *FAM46C* expression levels were reduced in all 5 cell lines. Copy number alterations were observed in AGS, N87, MKN74, GCIY, NUGC2 and MKN45 cell lines. Among them, *FAM46C* expression levels were decreased in MKN74, GCIY, NUGC2 and MKN45. However, hypermethylation in the promoter region was not detected (Fig. 1c).

FAM46C mRNA Expression Levels and Copy Number Alterations in Clinical Samples

The patient population included 89 males and 40 females with a mean age of 65.2 (range 26–83) years old. The median follow-up period was 50.6 (range 3.5–153.0) months. Of them, 37 patients (29%) experienced postoperative recurrences and the initial recurrent sites included peritoneum only (n=12), liver only (n=8), distal lymph node only (n=12), meningis (n=1), ovary (n=1), peritoneum/liver (n=1), peritoneum/node (n=1) and node/bone (n=1). Figure 2 shows *FAM46C* mRNA expression levels in cancerous compared with non-cancerous tissues from 129 patients. In most patients (117/129, 90.7%), *FAM46C* mRNA expression levels were decreased in cancerous

tissues.

Patients were stratified into high and low *FAM46C* expression groups (n=64 and 65, respectively), according to the median value of *FAM46C* mRNA levels in cancerous tissues from 129 patients. Macroscopic tumor size was significantly associated with *FAM46C* mRNA levels in GCs. Copy number alterations at *FAM46C* locus were observed in GC tissues of 45 (35%) patients. There was no significant difference in prevalence of the copy number alteration between the high and low *FAM46C* expression groups (Supplemental Table 2).

FAM46C Expression Level as a Prognostic Factor in GC Patients

Patients in the low *FAM46C* expression group were likely to have a shorter overall survival than those in the high expression group (5-year survival rates: 85% [95% confidence interval (CI): 70–93%] versus 60% [95% CI: 46–71%], P=0.003); Fig. 3a). Similar results were obtained for disease-free survival (3-year survival rates were significantly higher for the high expression group at 86% (95%CI: 74–92%) versus 60% (95%CI: 46–70%, $P = 0.001$) (Fig. 3b). In multivariable analysis, CA19-9 >37 IU/mL, positive vessel invasion and low *FAM46C* expression were identified as independent prognostic factors of recurrence-free survival. Moreover, *FAM46C* expression level had the highest hazard ratio at 4.61 (Table 2).

Subsequently, subgroup analysis of disease-free survival stratified by the UICC stages was performed. No patients with stage I GC had postoperative recurrences. A similar trend was observed

in both stages II and III. Surprisingly, no recurrences were found in patients with stage II GC and high *FAM46C* expression. Patients with stage II GC and low *FAM46C* expression were found to have lower survival rates similar to those with stage III GC and high expression (3-year survival rates: 60% [95%CI: 32–80%] and 67% [95% CI: 47–82%],respectively, Fig. 3c). Moreover, *FAM46C* expression levels were low in all cases that were later found to have hepatic recurrence (Fig. 3d). On the other hand, there was no significant difference in the cumulative incidence of peritoneal between the high and low *FAM46C* expression groups (Supplemental Fig. 1). In patients with stage III GC, 30 (48%) of 62 patients experienced recurrences, and the recurrence rates in high and low *FAM46C* were 32% and 62%, respectively.

DISCUSSION

Here we present that reduced expression of *FAM46C* in GC tissues, identified using transcriptome analysis, is a potential biomarker for predicting GC recurrence after curative resection. Metastasis-specific transcriptome analysis allowed us to identify 21 candidate metastasis-specific genes, of which *FAM46C*, a previously unreported molecule in GC, was selected and underwent evaluation of the expression mechanisms after which its clinical implications were sought. *FAM46C* is a member of the *FAM46* family, of which each member's function is not well known.[18] Among them, however, *FAM46A*, which is associated with poly-A RNA binding (GO annotation) and retinitis pigmentosa, is an important paralog.[19,20] *FAM46C*, on chromosome 1p12, seems to enhance

replication of some viruses, or to play a role in the regulation of translation by acting as an mRNA stabilizing factor.[17,21] Mutations in *FAM46C*, which are frequently associated with copy number variants, have been reported in about 10% of multiple myeloma patients.[17,22,23] Additionally, patients with those mutations that coexist with polyploidy or homozygous deletions of *FAM46C*, reportedly have a poor prognosis.[17] Accordingly, three expression analysis assays were performed on GC cell lines *in vitro* to detect the genetic and epigenetic aberrations at the *FAM46C* locus. Furthermore, prognostic significance of *FAM46C* expression levels in GC tissues among patients who underwent R0 resection was assessed in an *ex vivo* study.

While eleven GC cell lines expressed *FAM46C* to varying degrees, point mutations in the *FAM46C* gene were detected in five GC cell lines accompanied with reduced levels of *FAM46C* transcription. Copy number alterations were found in six GC cell lines, four in which point mutations were detected as above. However, hypermethylation in the *FAM46C* promoter region was not detected. These findings indicated that DNA methylation has no regulatory roles in *FAM46C* transcription in GC and led us to discuss on influences of copy number alterations and point mutations on *FAM46C* transcription. Correlation between copy number alterations and point mutations have been elucidated recently; mutations were generated concomitantly with *de novo* complex genomic rearrangement events such as intrachromosomal duplication.[24] Such tandem alleles can also reduce processivity, which can lead to reduced expression.[24] Although mutations detected in this study were silent, it can be reasonably expected that complex gene rearrangements as above and

other mutations undetected in this study affecting protein coding could occur at the *FAM46C* locus, and might have occurred in GC tumor samples with reduced *FAM46C* expression, though this has yet to be proven.

Reduced *FAM46C* mRNA expression levels did not correlate with any “classical” malignant clinicopathological indicators (such as UICC stage, vessel invasion or nodule metastasis) but only with macroscopic tumor size.[25] These findings highlight the predictive value of *FAM46C* expression because it can be a new approach to identify patients at risk of recurrence, independent of TNM staging. Strikingly, patients with stage II GC in the high *FAM46C* expression group had no recurrences. Risk stratification of patients with stage II/III GC is important for optimization of postoperative adjuvant therapy.[26,27] Data of FAM46C expression levels may be helpful for establishment of the next adjuvant strategy by selecting high-risk patients for whom intense treatments could be proposed. Moreover, our results indicated that *FAM46C* might be a powerful tool to predict hepatic recurrence. Hepatic metastases and recurrences in GC are currently a hot topic in the GC research community because a decreased prevalence of *Helicobacter pylori* infection and an increased prevalence of adenocarcinoma of the esophagogastric junction in Asian countries have resulted in an increased number of patients suffering from hepatic metastases and recurrences in GC.[3,28,29] In the future, *FAM46C* expression level analyses, mutation detection, and/or copy number analyses of the primary lesions may hopefully serve as a biomarker that can predict postoperative outcome so as to tailor the method of adjuvant treatment as well as the follow up..

A limitation of this study was that *ex vivo* retrospective data were used. Considering the relatively small sample size, the cutoff line proposed in this study is difficult to be directly used for the next step. To validate *FAM46C* so that it will have clinical utility, a prospective study to optimize the cutoff line and confirm our results will be necessary. In addition, molecular and functional aspects of *FAM46C*, such as *FAM46C* as a tumor suppressor, and whether and how gene rearrangements such as mutations and copy number alterations regulate the expression of FAM46C, are important issues that remain to be elucidated.

In conclusion, our results indicate that reduced expression of *FAM46C* mRNA is a putative biomarker that may be used to predict recurrence after curative resection in GC.

DISCLOSURE None.

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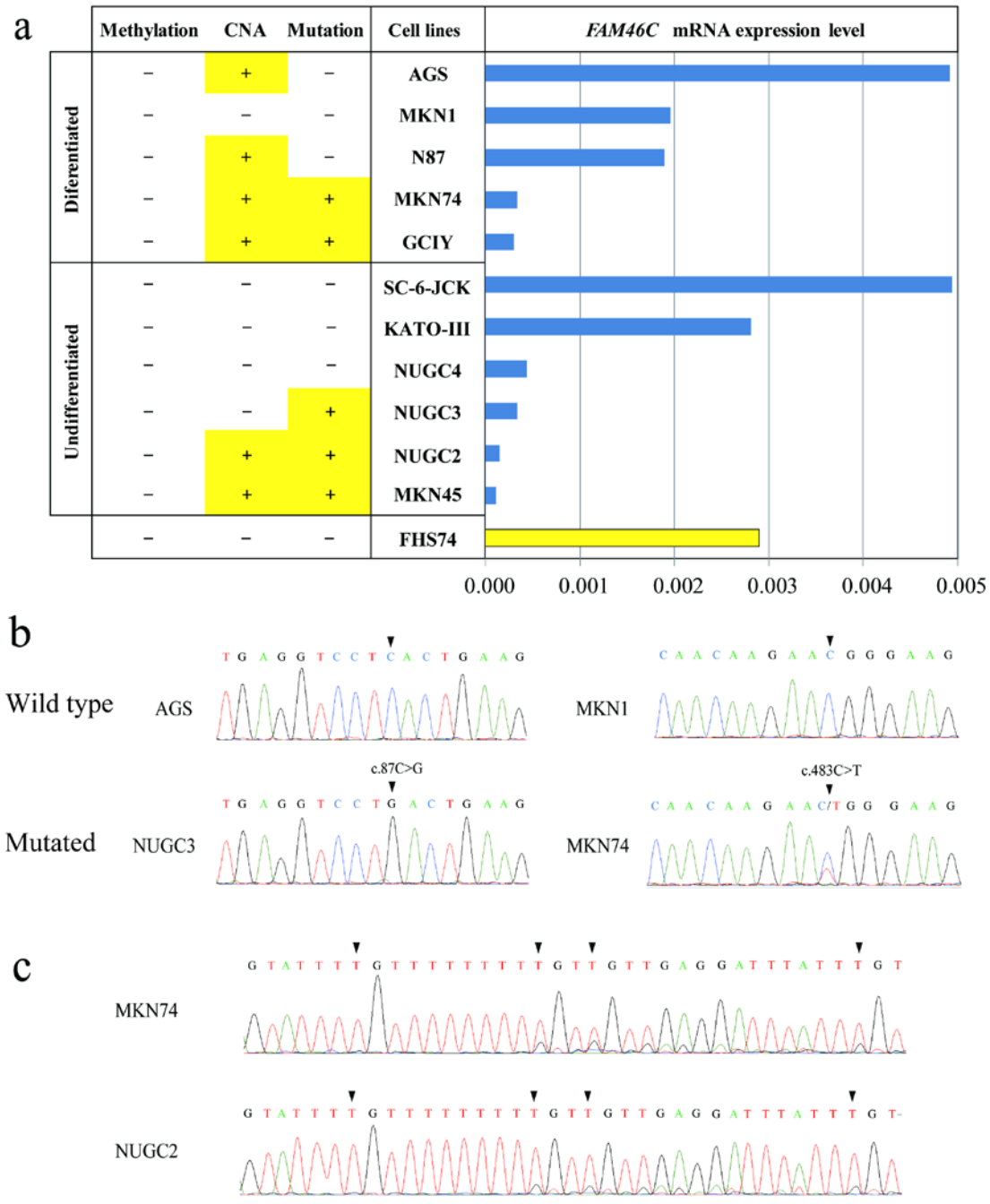


FIG. 1 a The level of *FAM46C* mRNA in 11 GC cell lines plus FHs74 *FAM46C* mRNA expression levels accompanied by a summary of assays exploring the mechanisms of reduced expression. **b** Mutational analysis of *FAM46C*, showing two representative mutations detected in NUGC3 and

MKN74 cell lines. **c** Methylation analysis of *FAM46C* using bisulfite sequencing of MKN74 and NUGC2 cell lines, indicating that any hypermethylation of the promoter region of *FAM46C* was not detected.

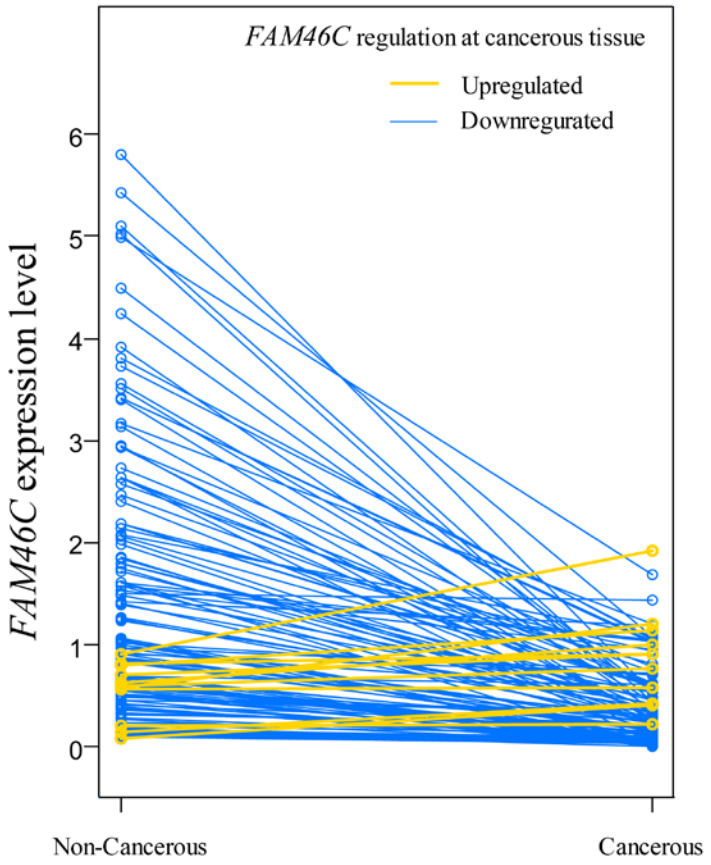


FIG 2 *FAM46C* mRNA expression levels in clinical samples. In 90.7% of patients (117/129), *FAM46C* mRNA expression levels decreased in cancerous tissues.

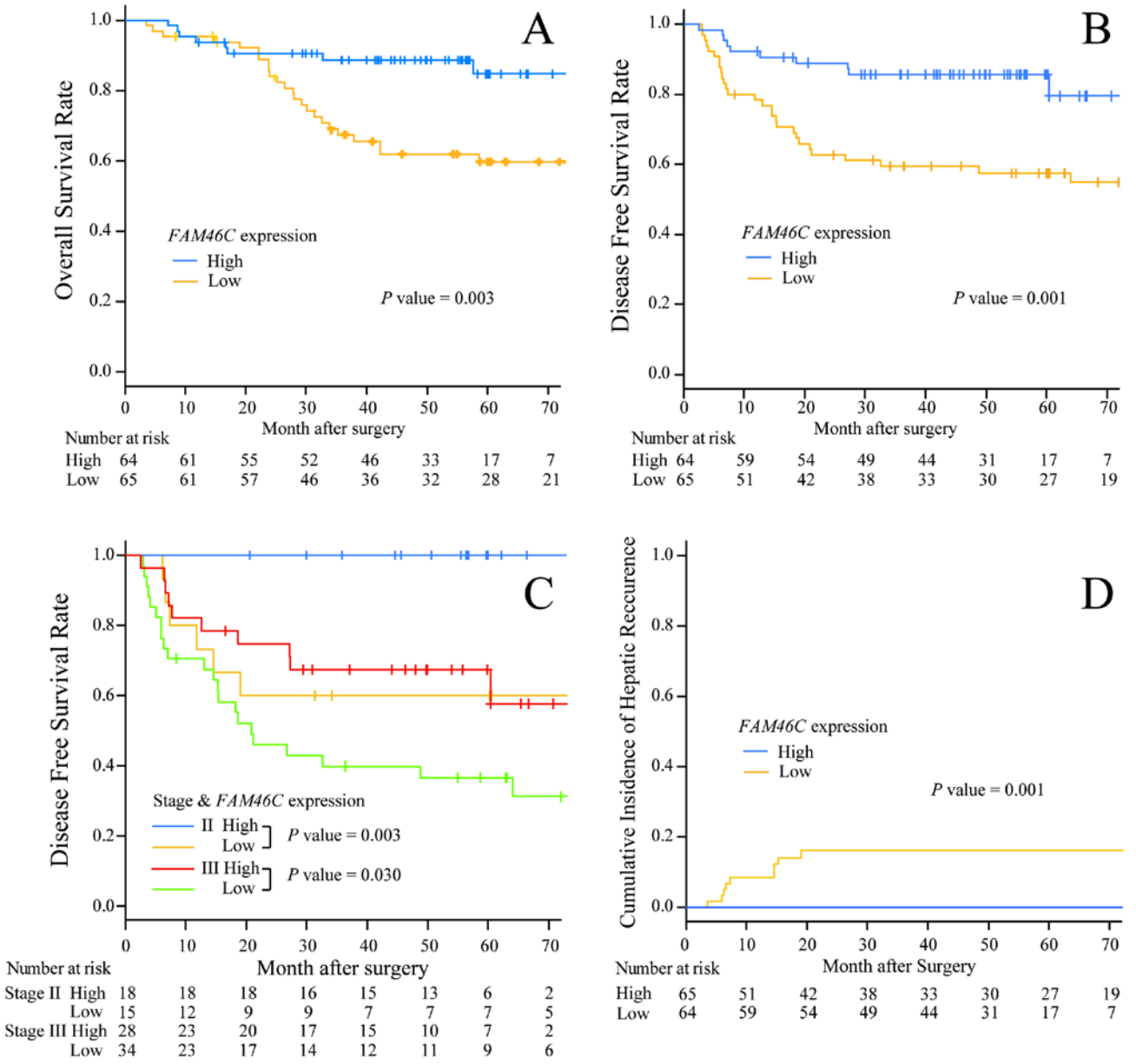
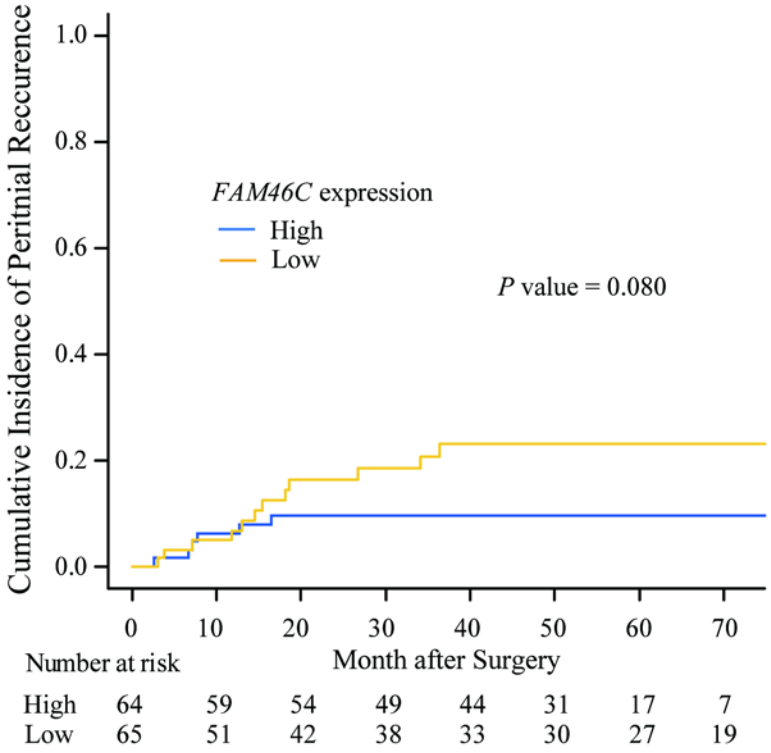


FIG. 3 Survival curve of GC patients stratified according to *FAM46C* expression levels. Patients in the low *FAM46C* expression group were likely to have a shorter overall survival (**a)** and disease-free survival (**b**) than those in the high expression group. (**c)** Patients in the low *FAM46C* expression group were likely to have shorter survival than those in the high expression group with stage II and III GC, respectively. (**d)** All the hepatic recurrences were observed in the low *FAM46C* expression group.



Supplementary FIG. 1 Peritoneal recurrences in the high and low *FAM46C* expression groups.

TABLE 1 List of candidate genes downregulated in gastric cancer tissues from patients with hepatic metastasis

Functional Category	Symbol	GC tissue /Normal		Full name	Location
		Log2	*P* value		
Transcription factor	*FAM46C*	-2.03	<0.001	family with sequence similarity 46, member C	1p12
	BTG2	-1.83	<0.001	BTG family, member 2	1q32
	IRF4	-2.92	<0.001	interferon regulatory factor 4	6p25-p23
	PAIP2B	-2.50	<0.001	poly(A) binding protein interacting protein 2B	2p13.3
	POU2AF1	-2.93	<0.001	POU class 2 associating factor 1	11q23.1
Metabolic enzyme	*ALDH3A1*	-3.40	<0.001	aldehyde dehydrogenase 3 family, member A1	17p11.2
	ASAH2	-2.82	<0.001	N-acylsphingosine amidohydrolase 2	10q11.21
	AKR1B10	-2.57	<0.001	aldo-keto reductase family 1, member B10	7q33
	GPAT3	-2.26	<0.001	glycerol-3-phosphate acyltransferase 3	4q21.23
	GSTA1	-2.76	<0.001	glutathione S-transferase alpha 1	6p12.1
	HRASLS2	-4.26	<0.001	HRAS-like suppressor 2	11q12.3
	XYLT2	-2.09	<0.001	xylosyltransferase II	17q21.33
Cytokine receptor	*LIFR*	-2.59	<0.001	leukemia inhibitory factor receptor alpha	5p13-p12
	IL7R	-2.02	<0.001	interleukin 7 receptor	5p13
Methyltransferase	*METTL7A*	-2.09	<0.001	methyltransferase like 7A	12q13.12
Proto-oncogene	*PIM2*	-2.43	<0.001	Pim-2 proto-oncogene, serine/threonine kinase	Xp11.23
Immunoglobulin mediator	*MZB1*	-2.71	<0.001	marginal zone B and B1 cell-specific protein	5q31.2
Multi-pass membrane protein	*GPR155*	-4.43	<0.001	G protein-coupled receptor 155	2q31.1
Membrane transporter	*MFSD4*	-4.32	<0.001	major facilitator superfamily domain containing 4	1q32.1
Signal transducer	*SLC9A4*	-3.95	<0.001	solute carrier family 9, subfamily A, member 4	2q12.1
Extracellular matrix protein	*DPT*	-3.22	<0.001	dermatopontin	1q12-q23

GC tissue: primary gastric cancer tissue, Normal: corresponding adjacent normal gastric tissue.

TABLE 2 Predictive factors of recurrence for 129 patients with R0 resection of gastric cancer

	Univariate			**Multivariate**		
	Hazard ratio	**95% CI**	***P* value**	**Hazard ratio**	**95% CI**	***P* value**
Age >65 years old	1.24	0.64–2.40	0.525			
Male sex	0.94	0.48–0.87	0.866			
CA19-9 >37 IU/mL	3.17	1.57–6.42	0.001	2.18	1.00–4.74	0.049*
CEA >5 ng/mL	1.48	0.65–3.37	0.346			
Location (Lower third)	0.75	0.39–1.48	0.410			
Tumor size >50 mm	2.56	1.27–5.17	0.009	1.55	0.76–3.18	0.230
Macroscopic Type 4 or 5	1.44	0.60–3.46	0.412			
Differentiated	0.70	0.36–1.36	0.290			
Lymphatic involvement	9.42	1.29–68.8	0.027	0.98	0.11–8.75	0.983
Vessel invasion	4.61	2.03–10.48	<0.001	3.92	1.58–9.73	0.003*
Invasive growth	2.13	1.12–4.06	0.022	1.97	0.91–4.27	0.087
Low *FAM46C* expression	3.13	1.52–6.46	0.002	4.61	2.15–9.89	<0.001*
T4	2.40	1.27–4.56	0.007	1.09	0.47–2.50	0.845
N1–3	10.14	3.11–33.02	<0.001	3.46	0.84–14.17	0.085
UICC stage III	7.15	2.99–17.13	<0.001	2.19	0.68–7.00	0.188

*Statistically significant (p value <0.05); CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; UICC, Union for International Cancer Control; CI, Confidential interval.

Supplemental TABLE 1 Specific primers for polymerase chain reaction assays

Target	Assay	Forward	Reverse
FAM46C	Quantitative RT-PCR	CATGTGGCTCTTCCAACAGA	CTTCAGCTCCACGTTCTTCC
	Direct Sequencing 1	GCTTCTCACCCCTCACTTTCA	GCACTTTGATGCCTGCCTCC
	Direct Sequencing 2	CGTCCAGACCGTCCGCAGT	CAGAAGGGAACACAGAACCAC
	Direct Sequencing 3	CAGAGGCAGAATTTCAGCTGG	CAAACTTCAGCTCCACGTTCTT
	Direct Sequencing 4	CCTGATCTCCCTCTCCAACAA	GAAGTGCTCAGAGATGGGATTA
	Direct Sequencing 5	GATTCTTTGCTTTTCTTCTATGAC	CACATGTAGCGCTCTAGAGTTT
	Direct Sequencing 6	CCCACAGACCAGGAAGAAATC	CACGCAAGGCCAGGAGGGA
	Direct Sequencing 7	CAGGCAGACTCTGAACCTCAT	CCTAGCCCTATTGGGGTTCC
	Bisulfite sequence	AAAAAGAAAGGTGTTTTATTAATTT	AAAACTACCCTCTAATTAACTCTTC
GAPDH	Quantitative RT-PCR	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

FAM46C, family with sequence similarity 46, member C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription polymerase chain reaction.

Supplementary TABLE 2 Association between *FAM46C* mRNA level and clinicopathological factors of 129 patients

	High *FAM46C*	Low *FAM46C*	*P* value
Age (year)			
<65	28	25	0.594
≥65	36	40	
Sex			
Male	43	46	0.706
Female	21	19	
CEA (ng/mL)			
≤5	53	57	0.467
>5	11	8	
CA19-9 (IU/mL)			
≤37	57	53	0.321
>37	7	12	
Tumor Location			
Entire	3	2	0.905
Upper third	16	15	
Middle third	21	20	
Lower third	24	28	
Tumor Size (cm)			
<50	38	24	0.014*
≥50	26	41	
Tumor Type			
Type 4 or 5	10	5	0.181
Others	54	60	
Tumor invasion (T, UICC)			
T1-3	39	41	0.857
T4	25	24	
Lymph node Metastasis (N, UICC)			
absent	25	27	0.858
present	39	38	
Pathological differentiation			
Differentiated	42	33	0.109
Undifferentiated	22	32	
Lymphatic involvement			
negative	12	10	0.646
positive	52	55	
Vessel invasion			
negative	29	29	1.000
positive	35	36	
Growth type			
expansive	49	16	0.437
invasive	44	20	
UICC stage			
I	18	16	0.632

II	18	15	
III	28	34	
Copy number alteration at FAM46C locus			
absent	43	41	0.624
present	21	24	

*Statistically significant (*P* value <0.05); CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; UICC, Union for International Cancer Control