Received: 28 April 2018 | Accepted: 1 August 2018
DOI: 10.1002/jso.25217

RESEARCH ARTICLE

High stromal transforming growth factor β–induced expression is a novel marker of progression and poor prognosis in gastric cancer

Masaki Suzuki MD[1] | Takehiko Yokobori MD, PhD[1,2] | Navchaa Gombodorj MD[1] | Masakazu Yashiro MD[3] | Andrei Turtoi PhD[4,5,6,7] | Tadashi Handa MS[8] | Kyoichi Ogata MD, PhD[1] | Tetsunari Oyama MD, PhD[8] | Ken Shirabe MD, PhD[9] | Hiroyuki Kuwano MD, PhD, FACS[1]

[1]Department of General Surgical Science, Gunma University Graduate School of Medicine, Maebashi, Japan

[2]Department of Innovative Cancer Immunotherapy, Gunma University Graduate School of Medicine, Maebashi, Japan

[3]Department of Surgical Oncology Molecular Oncology and Therapeutics, Osaka City University Graduate School of Medicine, Osaka, Japan

[4]Institut du Cancer, Montpellier, France

[5]INSERM U1194, Montpellier, France

[6]Institut de Recherche en Cancérologie de Montpellier, Montpellier, France

[7]Université Montpellier, Montpellier, France

[8]Department of Diagnostic Pathology, Gunma University Graduate School of Medicine, Maebashi, Japan

[9]Department of Hepatobiliary and Pancreatic Surgery, Gunma University Graduate School of Medicine, Maebashi, Japan

Correspondence
Masaki Suzuki, MD, Department of General Surgical Science, Graduate School of Medicine, Gunma University 3-39-22 Showa-Machi, Maebashi 371-8511, Japan.
Email: masakick11@gmail.com
Takehiko Yokobori, MD, PhD, Department of Innovative Cancer Immunotherapy, Graduate School of Medicine, Gunma University 3-39-22, Showa-Machi, Maebashi, 371-8511, Japan.
Email: bori45@gunma-u.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Numbers: JP15K10085, JP26461969, JP17K19893

Background and Objectives: Transforming growth factor β-induced (TGFBI) protein is a secreted extracellular matrix protein with conflicting roles in cancer, acting as a tumour suppressor and a promoter, which appears to be tissue specific. The role of TGFBI in gastric cancer (GC) remains unclear, which we aimed to investigate using the clinical samples as well as an in vitro coculture model of GC.

Methods: The clinical significance of TGFBI was assessed in 208 GC samples using immunohistochemistry. Molecular function of TGFBI in the GC cells was examined by small interfering RNA-mediated TGFBI downregulation in the gastric fibroblasts cocultured with the GC cells.

Results: TGFBI expression was localised mainly in the cancer stroma and not in the noncancerous gastric tissue or the GC cells. High TGFBI expression was significantly associated with poor prognosis and cancer progression. Downregulation of TGFBI in the cocultured gastric fibroblasts inhibited the invasion and migration abilities of the GC cells.

Conclusions: High stromal TGFBI expression might be a useful predictive marker for poor prognosis in GC patients. Furthermore, TGFBI in the cancer stromal cells is a promising target for GC treatment.

KEYWORDS
cancer-associated fibroblast, gastric cancer, stroma, transforming growth factor β-induced

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1 | INTRODUCTION

Gastric cancer (GC) is a leading cause of malignancy-related death globally.[1] Despite improvements in the treatment outcomes, GC prognosis is generally poor, with a 5-year relative survival rate remaining below 30% in most countries.[2] Surgical resection is the only treatment with curative intent, whereas postoperative chemotherapy was observed to improve GC prognosis after surgery[3]; however, most GC patients are not eligible for radical surgery due to the locally advanced or metastatic disease.[4] Thus, the identification of novel useful prognostic markers and therapeutic targets for GC treatment is essential.

Transforming growth factor β-induced (TGFBI) protein is a secreted extracellular matrix protein consisting of 683 amino acids and includes four evolutionarily conserved fasciclin-1 domains and a C-terminal Arg-Gly-Asp motif.[5] TGFBI was first described in a human lung adenocarcinoma cell line in 1992 as a protein that was inducible by transforming growth factor β (TGFβ),[6] which was confirmed in various human cell types since its discovery.[7,8] TGFBI was reported to play important roles in various clinical conditions such as malignancy, diabetes, and corneal dystrophy.[9-11]

In malignancies, TGFBI was revealed to perform conflicting roles, acting both as a tumour suppressor and a promoter. TGFBI downregulation was observed in leukaemia,[12] whereas hypermethylation of the TGFBI promoter, which suppresses TGFBI expression, was observed in the ovarian, prostate, and lung carcinomas.[13,14] These findings ascribe certain tumour-suppressor functions to TGFBI. Conversely, TGFBI was also known to exert tumour-promoter functions in various cancers. TGFBI upregulation was reported in pancreatic carcinoma and oral squamous cell carcinoma,[7,15] whereas in renal cell carcinoma, TGFBI expression was revealed as a promising prognostic marker.[16] Regarding GC, the transgenic mice overexpressing TGFBI developed spontaneous gastric adenocarcinoma at a high rate than wild-type mice[9]; however, few studies investigated the role of TGFBI in human GC specimens in detail.

The purpose of this study was to clarify the significance of TGFBI expression and its function in the clinical GC samples. We examined TGFBI expression by immunohistochemistry in 208 GC specimens and evaluated the relationship of TGFBI with clinicopathological factors. Moreover, we assessed whether the small interfering RNA (siRNA)-mediated TGFBI suppression in the gastric fibroblast cell lines affected the invasion and migration abilities of the cocultured human GC cells.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

Clinical samples collected from 208 GC patients (146 men and 62 women), who underwent surgery at the Department of General Surgical Science of Gunma University between 1999 and 2006, were included in this study. These samples which were used after an informed consent was obtained in accordance with institutional guidelines and the Declaration of Helsinki. Pathological features of the specimens were classified based on the 3rd English edition of the Classification of Gastric Carcinoma by the Japanese Gastric Cancer Association.[17]

2.2 | Immunohistochemistry

Sections of 4 μm were cut from the formalin-fixed, paraffin embedded GC sample blocks. Sections were mounted on the glass slides, deparaffinised in xylene, rehydrated in graded ethanol washes, and incubated in 0.3% hydrogen peroxide for 30 minutes at room temperature to block the endogenous peroxidase activity. Sections were then heated in boiled water containing Immunosaver (Nishin EM, Tokyo, Japan) at 98 to 100°C for 45 minutes. Nonspecific binding sites were blocked by incubation with Protein Block Serum-Free (Dako, Santa Clara, CA) for 30 minutes. Sections were then incubated with a TGFBI-specific antibody (Proteintech, Chicago, IL) at 1:100 dilution at 4°C for 24 hours. The primary antibody was visualised using the Histofine Simple Stain MAX-PO (Multi) kit (Nichirei, Tokyo, Japan), according to the manufacturer's instructions. The chromogen 3,3-diaminobenzidine tetrahydrochloride was applied as a 0.02% solution in 50 mM ammonium acetate-citrate acid buffer (pH 6.0) containing 0.005% hydrogen peroxide. Sections were lightly counterstained with haematoxylin and mounted. Specimens used as negative controls were incubated without the primary antibody to ensure that no detectable staining was evident.

TGFBI immunostaining was evaluated independently by two evaluators who were blinded to the patient data. We focused on the stromal TGFBI expression, and the intensity was scored as follows: 0, no staining; 1+, weak staining; 2+, moderate staining; 3+, strong staining. GC patients were classified as low stromal TGFBI expression group (scores 0 or 1+) or high stromal TGFBI expression group (scores 2 or 3+; Figure 1).

2.3 | Cell lines

The human GC cell lines MKN7, MKN45, MKN74, and GCIY, as well as the gastric fibroblast cell lines CAF64, CAF61, and NF64, were used in this study. MKN7, MKN45, and MKN74 were purchased from the JCRB Cell Bank (Osaka, Japan), and GCIY was purchased from RIKEN BRC (Tokyo, Japan). All gastric cell lines were maintained in Roswell Park Memorial Institute 1640 medium (Wako, Osaka, Japan) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA). Gastric fibroblast cell lines were isolated from surgical specimens at Osaka City University. CAF64 and CAF61 originated from the fibroblasts of GC tissues, and NF64 originated from the normal gastric tissue; both cell lines were maintained in Dulbecco modified Eagle medium (DMEM; Wako) supplemented with 10% FBS and 1% penicillin-streptomycin (Invitrogen). Precise protocols used to establish the fibroblast cell lines were described in a previous report.[18] All cell lines were cultured in a humidified incubator with 5% CO_2 at 37°C.

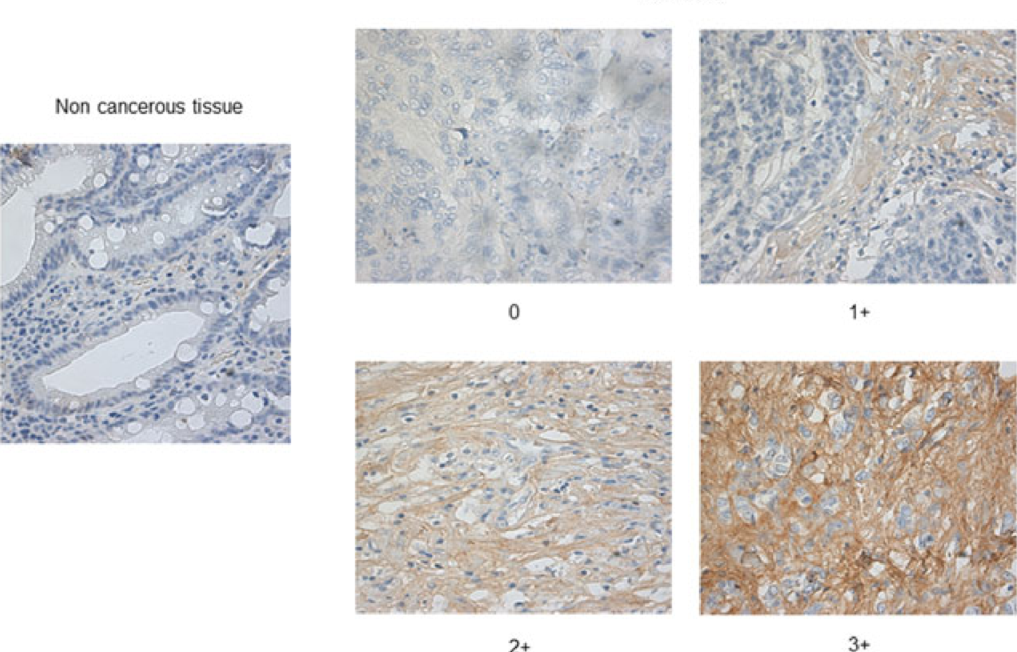

FIGURE 1 TGFβI expression in the gastric tissue. Representative immunohistochemical staining for TGFβI in the noncancerous gastric tissue and GC specimens (original magnification, ×400). GC, gastric cancer; TGFβI, transforming growth factor β-induced [Color figure can be viewed at wileyonlinelibrary.com]

2.4 | Protein extraction and Western blot analysis

Total protein was extracted from MKN7, MKN45, MKN74, GCIY, KMST6, CaF61, CaF64, and NF64 cells using lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol, 0.5% NP40, 400 mM NaCl, 4 μg/mL aprotonin, PMSF, proteasome inhibitor MG-132, and 1 mM DTT). Total protein (10 μg) was electrophoresed on 4% to 12% Bis-Tris Mini Gels (Life Technologies Corporation, Carlsbad, CA), and then electroblotted at 300 mA for 90 minutes on a nitrocellulose membrane (Invitrogen). Western blot analysis was used to confirm the expression of TGFBI and TGF-β1 proteins: these proteins were detected using anti-TGFBI rabbit monoclonal antibody (1:300; Proteintech; catalogue no. 10188-1-AP), anti-TGF-β1 mouse monoclonal antibody (1:1000; Cambridge, UK, catalogue no. ab27969), and HSC70 mouse monoclonal antibody (1:1000; Santa Cruz Biotechnology, Dallas, TX; catalogue no. sc-7298). HSC70 expression was used as a loading control. The signals were detected using the ECL Western blot analysis Detection System (GE Healthcare Life Sciences) and an Image Quant LAS 4000 machine (GE Healthcare Life Sciences).

2.5 | RNA interference for knockdown of TGFBI

TGFBI-specific siRNAs 1 and 2 were purchased from Theoria Science (Tokyo, Japan). CAF64 cells were plated at a density of 1.0×10^6 cells per well in 100 μL Opti-MEM I Reduced Serum Medium (Invitrogen). TGFBI-specific siRNAs 1 and 2 and a negative control siRNA were added to the cells at a concentration of 10 nM, and siRNA transfection was achieved using a CUY-21 EDIT II electroporator (BEX, Tokyo, Japan), according to the manufacturer's instructions. One poring pulse was applied at 125 V (pulse length, 10.0 ms; pulse interval, 40.0 ms), and five transfer pulses were applied at 10 V (pulse length, 50.0 ms; pulse interval, 50.0 ms). After 72-hour incubation, downstream experiments were performed.

2.6 | Cell invasion assay

Invasion ability of MKN7 and MKN45 cells was analysed using Matrigel-coated transwell chambers with an 8-μm pore size (Corning, New York, NY). CAF64 cells (2.0×10^5) were seeded in 24-well plates in DMEM containing 10% FBS, MKN7 (5.0×10^4), and MKN45 (2.5×10^4) cells were seeded in serum-free media in the upper chamber of the Matrigel-coated plates. After incubation for 24 hour (MKN7) or 48 hour (MKN45), the chambers were removed, washed with phosphate-buffered saline, and cleaned using a cotton swab. Cells were then fixed in methanol and stained with the Diff-Quick stain (Sysmex, Kobe, Japan). Membranes were cut and observed under ×100 magnification using bright field microscopy.

2.7 | Wound-healing assay

MKN7 (1.0×10^5), MKN45 (5.0×10^5), and CAF64 (5.0×10^4) cells were plated using a coculture model. Briefly, MKN7 and MKN45 cells were seeded in 24-well plates, and CAF64 cells were seeded in the upper chamber of the transwell plate with an 8-μm pore size (Corning). After MKN7 and MKN45 cells reached confluence, a scratch wound was made by creating a straight line using a sterile pipette tip. Cells were washed and incubated with DMEM containing 10% FBS. Closure of the wound was evaluated at 12 hour (MKN7)

and 24 hour (MKN45) after the wound creation at ×40 magnification using bright field microscopy.

2.8 | Statistical analysis

Statistically significant differences were analysed with Student *t* test for continuous variables and the χ^2 test for categorical variables. Analysis of variance (ANOVA) was used to assess the statistical significance of in vitro assays. When the results by ANOVA were significant, the Dunnett multiple comparisons test was used to assess the differences among groups. Kaplan-Meier curves were generated for overall survival regarding clinical data, and statistical significance was determined using the log-rank test. Univariate and multivariate survival analyses were performed using the Cox proportional hazards model. A *P* value of less than 0.05 was considered as statistically significant. All statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

3 | RESULTS

3.1 | Immunohistochemical staining for TGFBI in clinical GC specimens

TGFBI expression in 208 GC specimens was examined by immunohistochemistry using a tissue microarray. TGFBI expression was detected mainly in the cancer stroma and not in the GC cells or the noncancerous gastric tissue (Figure 1). Stromal TGFBI expression in the GC tissue was significantly higher than that in the noncancerous tissue ($P < 0.001$; Table 1).

3.2 | Clinicopathological significance of TGFBI expression in patients with GC

Among 208 GC cases, the stromal TGFBI expression scores were 0, 1+, 2+, and 3+ in 58 (27.9%), 69 (33.2%), 41 (19.8%), and 40 (19.2%) samples, respectively. About 127 (61.1%) samples were classified into the low TGFBI-expression group and 81 (38.9%) samples were classified into the high TGFBI-expression group.

The relationship of stromal TGFBI expression with clinicopathological factors in this cohort of 208 GC patients is presented in Table 2. High TGFBI expression was significantly associated with depth of tumour invasion ($P < 0.001$), lymphatic invasion ($P = 0.001$), venous invasion ($P = 0.046$), peritoneal dissemination ($P = 0.045$), and pathological stage ($P < 0.001$).

TABLE 1 Stromal TGFβI expression in noncancerous and cancer tissue

Stromal TGFβI expression	Noncancerous tissue, *n* = 46	GC tissue, *n* = 208	*P* value
0	26	58	<0.001*
1+	17	69	
2+	2	41	
3+	1	40	

Abbreviations: GC, gastric cancer; TGFβI, transforming growth factor β-induced.
*$P < 0.05$.

TABLE 2 Correlation analysis of TGFβI expression and clinicopathological factors in the study cohort of 208 patients with gastric cancer

	Stromal TGFβI expression		
Variables	Low, *n* = 127	High, *n* = 81	*P* value
Age	65.4 ± 10.8	63.4 ± 10.9	0.18
Sex			
Male	88	58	0.75
Female	39	23	
Histology			
Tubular	49	29	0.4
Poor	67	40	
Signet	6	9	
Other	5	3	
Depth			
sm, mp, ss	85	31	<0.001*
se, si	42	50	
Lymph node metastasis			
Absent	45	22	0.23
Present	82	59	
Lymphatic invasion			
Absent	18	1	0.001*
Present	109	80	
Venous invasion			
Absent	45	18	0.046*
Present	82	63	
Peritoneal dissemination			
Absent	123	73	0.045*
Present	4	8	
Stage			
I and II	75	26	<0.001*
III and IV	52	55	

Abbreviations: TGFβI, transforming growth factor β-induced.
*$p < 0.05$.

The Kaplan-Meier analysis of data from 208 GC patients demonstrated that the overall survival in the high TGFBI-expression group was significantly lower than that in the low TGFBI-expression group ($P = 0.0016$; Figure 2).

Univariate regression analyses for overall survival among the cohort of 208 GC samples indicated that the depths of tumour invasion ($P < 0.0001$), lymph node metastasis ($P < 0.0001$), lymphatic invasion ($P = 0.0014$), venous invasion ($P < 0.0001$), peritoneal dissemination ($P = 0.0008$), pathological stage ($P < 0.0001$), and TGFBI expression ($P = 0.0039$) were significantly associated with poor prognosis; however, multivariate analysis indicated that high TGFBI expression was not an independent predictor for poor prognosis (Table 3).

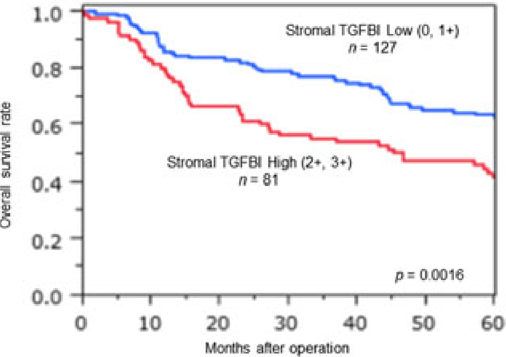

FIGURE 2 Kaplan-Meier curves for overall survival according to the level of TGFβI expression. Overall survival rate in the high TGFβI-expression group is significantly lower than that in the low TGFβI-expression group (P = 0.0016). TGFβI, transforming growth factor β-induced [Color figure can be viewed at wileyonlinelibrary.com]

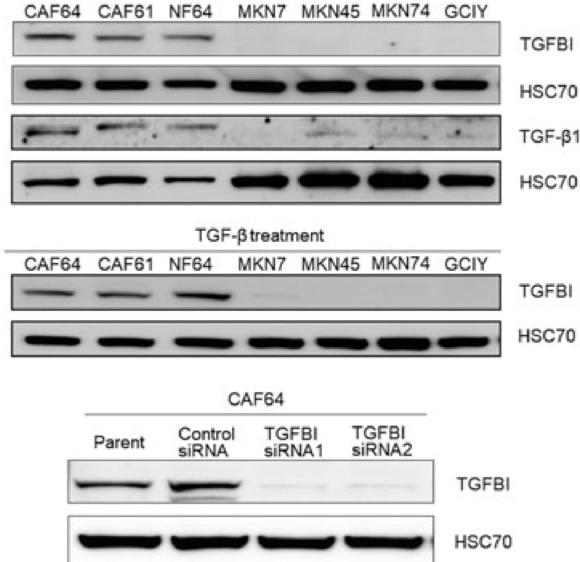

FIGURE 3 TGFβI and TGF-β1 protein expression in gastric fibroblast and GC cell lines. In upper panel, TGFβI and TGF-β1 expression is determined in CAF64, CAF61, and NF64 gastric fibroblast cell lines and MKN7, MKN45, MKN74, and GCIY GC cell lines by Western blot analysis. In middle panel, the induction of TGFβI after TGF-β1 treatment (5 ng/mL for 48 hours) is determined in gastric fibroblast and GC cell lines. In lower panel, TGFβI expression is knockdown in CAF64 cells transfected with a TGFβI siRNA. HSC70 is used as the loading control. GC, gastric cancer; siRNA, small interfering RNA; TGF-β1, transforming growth factor β1; TGFβI, transforming growth factor β-induced

3.3 | TGFBI and TGF-β1 expression in GC and gastric fibroblast cell lines

We evaluated the protein expression of TGFBI and TGF-β1 in CAF64, CAF61, and NF64 gastric fibroblast cell lines and MKN7, MKN45, MKN74, and GCIY GC cell lines by Western blot analysis (Figure 3, upper panel). TGFBI protein was detected in all fibroblast cell lines and in none of the GC cell lines. The expression of TGF-β1 in fibroblast cell lines was higher than GC cell lines. Furthermore, we evaluated TGFBI induction by TGF-β1 treatment. The expression of TGFBI was not induced in GC cell lines (Figure 3, middle panel).

TGFBI expression was higher in CAF64 cells than the other fibroblast cell lines. Therefore, the CAF64 cell line was used to clarify whether TGFBI in fibroblasts contributed to the malignant phenotype in two GC cell lines: MKN7 and MKN45, which were derived from well-differentiated and poorly-differentiated adenocarcinomas, respectively.

3.4 | CAF64 cells upregulated the invasion and migration abilities of cocultured MKN7 and MKN45 cells

To evaluate the effect of CAF64 cells on GC cells, we performed an invasion and wound-healing assay in a coculture model. The

TABLE 3 Univariate and multivariate analyses of the clinicopathological factors affecting the overall survival rate following surgery in gastric cancer

	Univariate analysis			Multivariate analysis		
Clinicopathological variables	**RR**	**95% CI**	***P* value**	**RR**	**95% CI**	***P* value**
Sex (male/female)	1.26	0.83-1.97	0.2907	–	–	–
Histology (differentiated/undifferentiated)*	1.36	0.90-2.07	0.1439	–	–	–
Depth (sm, mp, ss/se, si)	3.45	2.31-5.24	<0.0001*	1.86	1.15-3.08	0.0112*
Lymph node metastasis (absent/present)	2.74	1.70-4.65	<0.0001*	1.19	0.57-2.48	0.6476
Lymphatic invasion (absent/present)	4.32	1.63-17.6	0.0014*	1.58	0.50-6.99	0.4605
Venous invasion (absent/present)	2.47	1.53-4.19	<0.0001*	1.56	0.93-2.74	0.0909
Peritoneal dissemination (absent/present)	3.56	1.79-6.42	0.0008*	1.75	0.85-3.30	0.1216
Stage (I,II/III,IV)	4.09	2.65-6.52	<0.0001*	1.98	0.97-4.20	0.0604
TGFβI expression (low/high)	1.79	1.21-2.64	0.0039*	1.24	0.83-1.87	0.2981

Abbreviations: RR, relative risk; CI, confidence interval.
*$p < 0.05$.

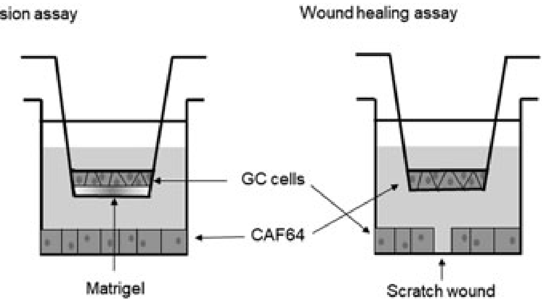

FIGURE 4 Schema for the coculture model used in this study. In the invasion assay, GC cells are seeded in the upper chamber coated with Matrigel, and CAF64 cells are seeded in the lower chamber. In the wound-healing assay, CAF64 cells are seeded in the upper chamber, and GC cells are seeded in the lower chamber, which were scratched. GC, gastric cancer

experimental paradigms are presented in Figure 4. The invasion ability of MKN7 and MKN45 cells was significantly upregulated when they were cocultured with CAF64 cells ($P = 0.0007$ and <0.0001, respectively; Figure 5, left panel). Similarly, the migration ability of MKN7 and MKN45 cells was also significantly upregulated upon coculturing with CAF64 cells ($P = 0.0003$ and <0.0001, respectively; Figure 6, left panel).

3.5 | TGFBI knockdown in CAF64 cells suppresses the invasion and migration abilities of the cocultured MKN7 and MKN45 cells

To evaluate the correlation between the TGFBI expression in CAF64 cells and the invasion and migration abilities of MKN7 and MKN45 cells, we performed TGFBI knockdown in our coculture model and used the invasion and wound-healing assay (Figure 4). First, the knockdown of TGFBI in CAF64 cells using siRNA was confirmed by Western blot analysis (Figure 3, lower panel). The CAF64-induced invasion ability of MKN7 and MKN45 cells was suppressed in the presence of CAF64 cells with TGFBI knockdown using siRNA ($P = 0.0002$, <0.0001; =0.0171, =0.0116) (Figure 5, right panel). CAF64-induced migration ability of MKN7 and MKN45 cells was also suppressed similar to that observed with the invasion assay ($P < 0.0001$; Figure 6, right panel).

4 | DISCUSSION

In this study, we found that TGFBI was expressed primarily in cancer stroma and not in the GC cells or noncancerous tissue. We also reported that high TGFBI levels in cancer stroma correlated with

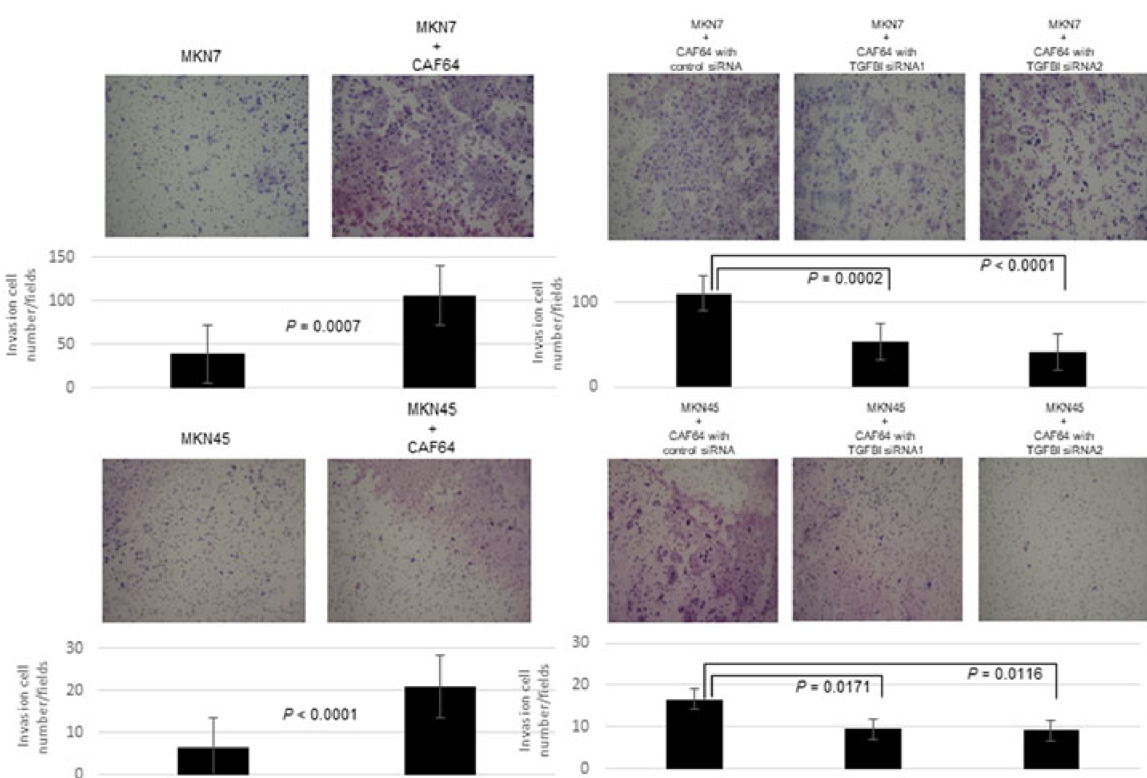

FIGURE 5 Effect of cocultured gastric fibroblast cell lines on the invasion abilities of human gastric cancer cell lines. Invasion ability of MKN7 and MNK45 cells is upregulated in the presence of cocultured CAF64 cells. However, it is downregulated by the cocultured CAF64 cells transfected with the TGFβI siRNA. Data are presented as mean ± SD. siRNA, small interfering RNA; TGFβI, transforming growth factor β-induced [Color figure can be viewed at wileyonlinelibrary.com]

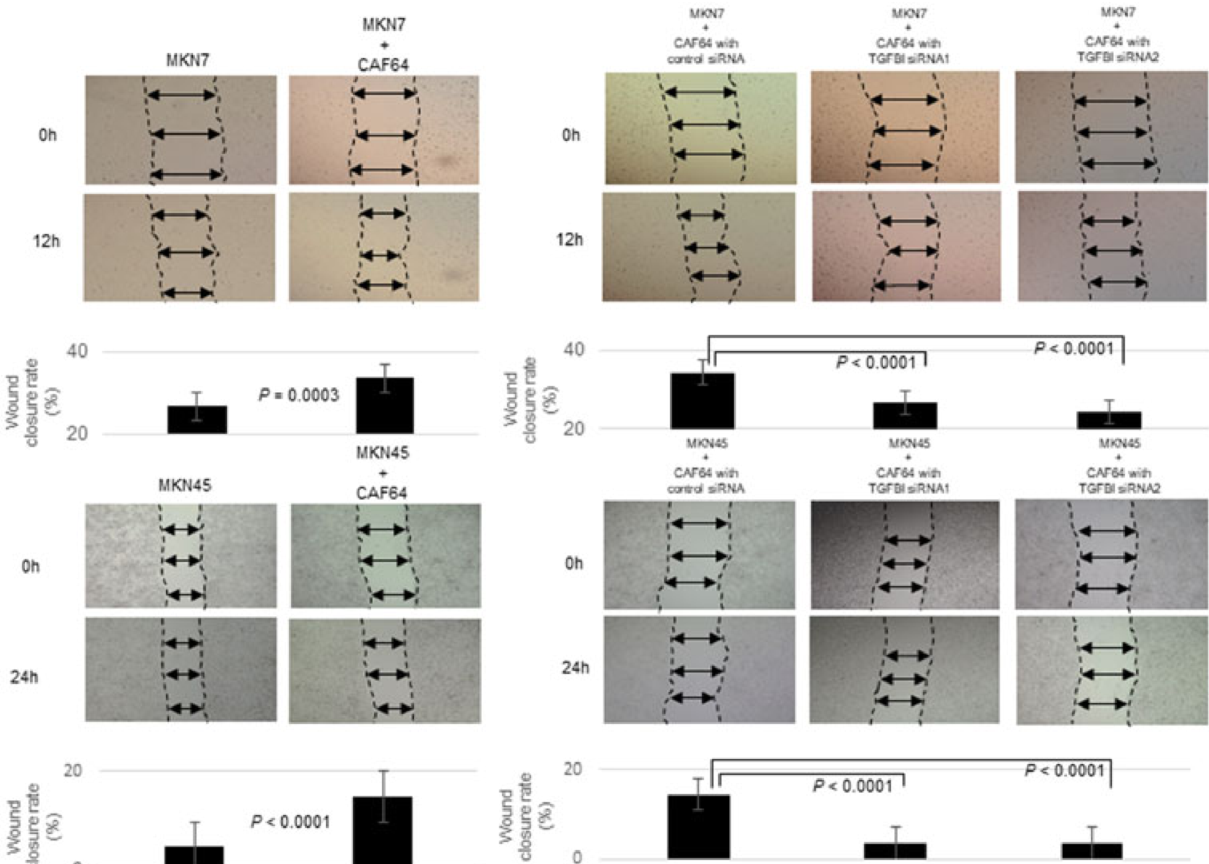

FIGURE 6 Effect of cocultured gastric fibroblast cell lines on the migration abilities of human gastric cancer cell lines. Migration ability of MKN7 and MNK45 cells is upregulated in the presence of cocultured CAF64 cells. However, it is downregulated by the cocultured CAF64 cells transfected with the TGFβI siRNA. Data are presented as mean ± SD. siRNA, small interfering RNA; TGFβI, transforming growth factor β-induced [Color figure can be viewed at wileyonlinelibrary.com]

cancer progression and poor prognosis in the clinical GC samples. Moreover, TGFBI knockdown in fibroblasts suppressed the invasion and migration ability of MKN7 and MKN45 cells in an in vitro coculture model.

TGFBI was initially described as a protein that was upregulated by TGF-β in the lung cancer cells.[6] Increased TGFBI expression in response to TGF-β was also demonstrated in the pancreatic cancer cells.[7] Ma et al[19] reported that increased TGFBI secreted by cancer cells was associated with high clinical grade in the colon cancers. Lauden et al[20] examined the function of TGFBI in melanoma using cell lines with high TGFBI expression and demonstrated that TGFBI was required for melanoma metastatic outgrowth. Although these reports suggest that TGFBI might be derived from the cancer cells, our assessment of GC tissue samples by immunohistochemistry revealed that GC cells did not express TGFBI. Moreover, we found that TGFBI was expressed in all gastric fibroblast cell lines and none of the GC cell lines included in the current study. Mizoi et al[21] previously reported that TGF-β signalling was significantly upregulated in the cancer stromal tissues in GC. TGF-β signalling was also shown to be activated in fibroblasts of GC tissues.[22] Moreover, Fuyuhiro et al[23] suggested that fibroblasts around GC were associated with aggressiveness of GC cells via the TGF-β signalling.[23] Therefore, we suggest that TGFBI might be derived from gastric stromal fibroblasts via activation of the TGF-β signalling, in contrast to the GC cells.

TGFBI has been observed to serve contradictory functions in tumorigenesis.[24] Although TGFBI was reported to function as a tumour suppressor in leukaemia and ovarian, prostate, and lung carcinomas,[12-14] the tumour-promoting role of TGFBI was indicated in renal cell carcinoma, pancreatic carcinoma, oral and oesophageal squamous cell carcinoma, and colon carcinoma.[7,1615,19,25] Furthermore, Han et al[9] reported a role for TGFBI in tumorigenesis of the gastrointestinal tract[9] by demonstrating that TGFBI overexpression increased the incidence of spontaneous tumours including gastric adenocarcinoma in transgenic mice. In this study, we reported that high stromal TGFBI expression correlated with cancer progression and poor prognosis in clinical GC samples, suggesting that stromal TGFBI was acting as a tumour promoter in gastric tissue. Moreover, TGFBI expression might be a useful marker in predicting cancer progression and poor prognosis in GC.

In the current study, high stromal TGFBI expression was significantly correlated with peritoneal dissemination. Peritoneal dissemination is widely recognised as the most important and difficult therapeutic target in progressive GC phenotype.[26] Although various strategies including systemic chemotherapy, intraperitoneal chemotherapy, hyperthermia,

and aggressive surgery have been used to control peritoneal dissemination, their clinical benefits are limited.[27,28] Intraperitoneal paclitaxel administration in GC patients with peritoneal dissemination has been recently gaining attention as intraperitoneal paclitaxel is expected to remain inside the peritoneal cavity due to its large molecular weight and fat solubility.[29] Paclitaxel is also used as a standard treatment in ovarian cancer.[30] Wang et al[31] reported that silencing TGFBI by hypermethylation of its promoter correlated with paclitaxel resistance in ovarian cancer.[31] Moreover, Ahmed et al[32] indicated that cancer stromal TGFBI induced micro-tubule stabilisation and sensitisation to paclitaxel in ovarian cancer. These findings suggest that GC patients with high TGFBI expression might have poor prognosis with peritoneal dissemination and that treatment with paclitaxel might control the cancer progression and improve prognosis in refractory GC patients with peritoneal dissemination associated with high TGFBI expression. Assessment of TGFBI expression in GC patients with peritoneal dissemination might be useful in determining the relevant adaptations to treatment regimens including taxane-class chemotherapeutic agents.

Our clinical data revealed that high stromal TGFBI expression was associated with cancer progression based on the significant associations with depth of tumour invasion, lymphatic invasion, venous invasion, peritoneal dissemination, and pathological stage. Moreover, our assessment regarding the effect of TGFBI expressed by fibroblasts on GC cells using the coculture model revealed that TGFBI in fibroblasts upregulated the invasion and migration abilities of the GC cells. Han et al[9] found that TGFBI activated FAK/AKT/mTOR pathways were associated with the promotion of cell survival. Lauden et al[20] indicated that TGFBI knockdown led to growth inhibition through defective cell cycle progression in the melanoma cells. Specifically, they demonstrated that TGFBI was a regulator of cyclins and cyclin-dependent kinases that control the progression of cell cycle and cancer aggressiveness in several cancer types including GC.[33] Based on these observations, we propose that TGFBI localised in the GC stroma might be a promising target for therapeutic intervention through its ability to inhibit the invasion and migration abilities and cell cycle progression of cancer cells.

5 | CONCLUSIONS

TGFBI expression in the cancer stromal cells was associated with poor prognosis and cancer progression in GC patients. In an in vitro model, TGFBI expressed by fibroblasts regulated the invasion and migration abilities of the GC cells, which might be related to cancer progression. TGFBI expression is a potentially useful predictive marker for poor prognosis in GC. Moreover, TGFBI in the cancer stromal cells is a promising therapeutic candidate to improve prognosis in the GC patients.

ACKNOWLEDGEMENTS

We thank Ms. Yukie Saito, Ms. Tomoko Yano, Ms. Yuka Matsui, Ms. Sayaka Okada, Ms. Kayoko Takahashi, Ms. Yuki Saka, Ms. Mizue Murata, Ms. Kanna Nakamura, Ms. Harumi Kanai, and Ms. Fumie Takada for their excellent assistance. The authors would like to thank Enago (www.enago.jp) for the English language review. This study was funded by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (grant nos JP26461969, JP15K10085, and JP17K19893).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Masaki Suzuki http://orcid.org/0000-0002-1503-6861
Takehiko Yokobori http://orcid.org/0000-0003-3284-4796
Tadashi Handa http://orcid.org/0000-0002-5696-2060

REFERENCES

1. Ferro A, Peleteiro B, Malvezzi M, et al. Worldwide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. *Eur J Cancer*. 2014;50:1330-1344.
2. Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. *Methods Mol Biol*. 2009;472:467-477.
3. Cao J, Qi F, Liu T. Adjuvant chemotherapy after curative resection for gastric cancer: a meta-analysis. *Scand J Gastroenterol*. 2014;49: 690-704.
4. Cervantes A, Rosello S, Roda D, Rodriguez-Braun E. The treatment of advanced gastric cancer: current strategies and future perspectives. *Ann Oncol*. 2008;19(Suppl 5):v103-v107.
5. Kawamoto T, Noshiro M, Shen M, et al. Structural and phylogenetic analyses of RGD-CAP/beta IG-H3, a fasciclin-like adhesion protein expressed in chick chondrocytes. *Biochim Biophys Acta*. 1998;1395:288-292.
6. Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD, Purchio AF. cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. *DNA Cell Biol*. 1992;11:511-522.
7. Schneider D, Kleeff J, Berberat PO, et al. Induction and expression of betaIG-H3 in pancreatic cancer cells. *Biochim Biophys Acta*. 2002;1588:1-6.
8. Nabokikh A, Ilhan A, Bilban M, et al. Reduced TGF-beta1 expression and its target genes in human insulinomas. *Exp Clin Endocrinol Diab*. 2007;115:674-682.
9. Han B, Cai H, Chen Y, et al. The role of TGFBI (betaIG-H3) in gastrointestinal tract tumorigenesis. *Mol Cancer*. 2015;14:64.
10. Han B, Luo H, Raelson J, et al. TGFBI (betaIG-H3) is a diabetes-risk gene based on mouse and human genetic studies. *Hum Mol Gen*. 2014;23:4597-4611.
11. Dighiero P, Niel F, Ellies P, et al. Histologic phenotype-genotype correlation of corneal dystrophies associated with eight distinct mutations in the TGFBI gene. *Ophthalmology*. 2001;108:818-823.
12. Li Z, Luo RT, Mi S, et al. Consistent deregulation of gene expression between human and murine MLL rearrangement leukemias. *Cancer Res*. 2009;69:1109-1116.
13. Kang S, Dong SM, Park NH. Frequent promoter hypermethylation of TGFBI in epithelial ovarian cancer. *Gynecol Oncol*. 2010;118:58-63.
14. Shah JN, Shao G, Hei TK, Zhao Y. Methylation screening of the TGFBI promoter in human lung and prostate cancer by methylation-specific PCR. *BMC Cancer*. 2008;8:284.

15. Tomioka H, Morita K, Hasegawa S, Omura K. Gene expression analysis by cDNA microarray in oral squamous cell carcinoma. *J Oral Pathol Med*. 2006;35:206-211.
16. Lebdai S, Verhoest G, Parikh H, et al. Identification and validation of TGFBI as a promising prognosis marker of clear cell renal cell carcinoma. *Urol Oncol*. 2015;33:69.e11-8.
17. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 3rd english edition. *Gastric Cancer*. 2011;14:101-112.
18. Fuyuhiro Y, Yashiro M, Noda S, et al. Upregulation of cancer-associated myofibroblasts by TGF-beta from scirrhous gastric carcinoma cells. *Br J Cancer*. 2011;105:996-1001.
19. Ma C, Rong Y, Radiloff DR, et al. Extracellular matrix protein betaIG-H3/TGFBI promotes metastasis of colon cancer by enhancing cell extravasation. *Genes Dev*. 2008;22:308-321.
20. Lauden L, Siewiera J, Boukouaci W, et al. TGF-beta-induced (TGFBI) protein in melanoma: a signature of high metastatic potential. *J Invest Dermatol*. 2014;134:1675-1685.
21. Mizoi T, Ohtani H, Miyazono K, Miyazawa M, Matsuno S, Nagura H. Immunoelectron microscopic localization of transforming growth factor beta 1 and latent transforming growth factor beta 1 binding protein in human gastrointestinal carcinomas: qualitative difference between cancer cells and stromal cells. *Cancer Res*. 1993;53:183-190.
22. Ishimoto T, Miyake K, Nandi T, et al. Activation of transforming growth factor beta 1 signaling in gastric cancer-associated fibroblasts increases their motility, via expression of rhomboid 5 homolog 2, and ability to induce invasiveness of gastric cancer cells. *Gastroenterology*. 2017;153:191-204.e16.
23. Fuyuhiro Y, Yashiro M, Noda S, et al. Cancer-associated orthotopic myofibroblasts stimulates the motility of gastric carcinoma cells. *Cancer Sci*. 2012;103:797-805.
24. Ween MP, Oehler MK, Ricciardelli C. Transforming growth factor-beta-induced protein (TGFBI)/(betaIG-H3): a matrix protein with dual functions in ovarian cancer. *Int J Mol Sci*. 2012;13:10461-10477.
25. Ozawa D, Yokobori T, Sohda M, et al. TGFBI expression in cancer stromal cells is associated with poor prognosis and hematogenous recurrence in esophageal squamous cell carcinoma. *Ann Surg Oncol*. 2016;23:282-289.
26. Yamashita K, Sakuramoto S, Kikuchi S, Katada N, Kobayashi N, Watanabe M. Surgical resection of stage IV gastric cancer and prognosis. *Anticancer Res*. 2007;27:4381-4386.
27. Sugarbaker PH, Yonemura Y. Clinical pathway for the management of resectable gastric cancer with peritoneal seeding: best palliation with a ray of hope for cure. *Oncology*. 2000;58:96-107.
28. Fujimoto S, Takahashi M, Kobayashi K, et al. Relation between clinical and histologic outcome of intraperitoneal hyperthermic perfusion for patients with gastric cancer and peritoneal metastasis. *Oncology*. 1993;50:338-343.
29. Kono K, Yong WP, Okayama H, et al. Intraperitoneal chemotherapy for gastric cancer with peritoneal disease: experience from Singapore and Japan. *Gastric Cancer*. 2017;20:122-127.
30. Matei D. Novel agents in ovarian cancer. *Expert Opin Invest Drugs*. 2007;16:1227-1239.
31. Wang N, Zhang H, Yao Q, Wang Y, Dai S, Yang X. TGFBI promoter hypermethylation correlating with paclitaxel chemoresistance in ovarian cancer. *J Exp Clin Cancer Res*. 2012;31:6.
32. Ahmed AA, Mills AD, Ibrahim AEK, et al. The extracellular matrix protein TGFBI induces microtubule stabilization and sensitizes ovarian cancers to paclitaxel. *Cancer Cell*. 2007;12:514-527.
33. Wang Q, Li M, Zhang X, et al. Upregulation of CDK7 in gastric cancer cell promotes tumor cell proliferation and predicts poor prognosis. *Exp Mol Pathol*. 2016;100:514-521.

How to cite this article: Suzuki M, Yokobori T, Gombodorj N, et al. High stromal transforming growth factor β–induced expression is a novel marker of progression and poor prognosis in gastric cancer. *J Surg Oncol*. 2018;118:966-974. https://doi.org/10.1002/jso.25217