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IGFBP1 Is a Predictive Factor for Haematogenous Metastasis in Patients With Gastric Cancer

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Abstract. *Background/Aim: The clinicopathological significance and prognostic value of insulin-like growth factor binding protein 1 (IGFBP1) in gastric cancer have not been investigated to date. This study aimed to investigate the relationship of IGFBP1 expression with clinicopathological variables and prognosis. Materials and Methods: The correlation of IGFBP1 expression with the clinicopathological factors and the correlation of clinicopathogical factors with haematogenous metastasis in 219 gastric cancer patients who underwent surgery was examined. Results: High IGFBP1 expression was significantly associated with a poorer disease-specific survival (p<0.001) and relapse-free survival (p<0.001) in univariable analysis although IGFBP1 was not an independent prognostic factor. High IGFBP1 expression was the only independent risk factor of haematogenous metastasis. Conclusion: High IGFBP1 expression was associated with haematogenous metastasis and poor survival. IGFBP1 might become a new prognostic factor and a target of molecular targeted therapy of gastric cancer.*

Gastric cancer is the fifth most common type of malignant tumour worldwide. An estimated one million new cases are diagnosed every year, resulting in about 700,000 annual deaths (1). Surgical resection and subsequent adjuvant chemotherapy have become the standard treatment for most patients with clinically diagnosed locally advanced gastric cancer (2). Although chemotherapy including pre- and postoperative chemotherapy has been developed to prevent and control metastatic recurrence that is strongly related to gastric cancer mortality, the prognosis in patients with advanced tumour remains unsatisfactory (3, 4). Therefore, it is crucial to identify reliable prognosis markers that will allow better management and identification of potential therapeutic targets.

The insulin-like growth factor (IGF) axis plays a key role in the growth, differentiation and proliferation of mammalian cells or angiogenic activities and consists of two growth factors (IGF-I and IGF-II), their receptors (IGF-IR and IGF-IIR) and a group of insulin-like growth factor binding proteins (IGFBPs). IGFBPs consist of 7 well-characterized members (IGFBP1-7) (5). Matsubara *et al*. have reported that IGF-IR expression in gastric cancer specimens was a significant predictor of poor survival in patients with advanced gastric cancer (6). Anti-IGF-IR strategies may prove valuable in such patients. The actions of IGFs may be modulated by the IGFBPs in a positive or negative way, depending on the tissue type and the physiological/pathological status (7). In most circumstances, they inhibit IGF actions by preventing the binding to IGF receptors; however, they may also potentiate their actions (8). In gastric cancer, clinical impacts of IGFBP expression have been investigated for few subtypes of the IGFBPs family. The clinicopathological significance of IGFBP2 (9, 10) and IGFBBP7 (11, 12) in gastric cancer has already been reported, and IGFBP2 and IGFBP7 might become new prognostic factors in gastric cancer patients in the future.

In contrast, the clinicopathological significance and prognostic value of IGFBP1 in gastric cancer have not been investigated previously. IGFBP1 expression has been documented to be both, positively and negatively correlated with cancer risk, tumour progression and prognosis in colorectal cancer (13), breast cancer (14) and hepatocellular cancer (15). In the present study, the relationship of IGFBP1 expression with clinicopathological variables and prognosis in gastric cancer was examined.

Patients and Methods

Patients. The study group comprised 219 patients with gastric cancer who underwent surgery from January 2003 to December 2007 at the Department of Gastric Surgery, Tokyo Medical and Dental University. Each tumour was classified as per the tumour-

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node-metastasis (TNM) system recommended by the International Union against Cancer.

Patient characteristics are shown in Table I. Of the 219 patients, 165 were men and 54 were women. Their median age was 66 years (range=21-92 years). All patients were evaluated for recurrent disease using diagnostic imaging (computed tomography, ultrasonography, magnetic resonance imaging, and endoscopy) every 3-6 months. Patients with distant metastatic or recurrent disease received chemotherapy with S-1 alone or combined chemotherapy. Nineteen patients (9%) received adjuvant chemotherapy with S-1 after radical resection. All the patients were followed up until January 2013. The median follow-up duration was 60 months (range=3-111 months). Among all the patients, 18 had haematogenous metastasis (simultaneous metastasis: 1 patient, metachronous metastasis: 17 patients). Peritoneal metastasis was present in 43 patients (19.6%), and distant lymph node metastasis was observed in 32 patients (14.6%). Metastases to the liver, lung, brain and bone were defined as haematogenous metastases. Total 77 (35%) patients died, 67 (31%) had recurrent disease and 9 (4%) died because of other causes.

Human rights statement and informed consent. All procedures followed were in accordance with the ethical standards of the institutional review board of Tokyo Medical and Dental University (approval no. 831) and national ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, and later versions. Informed consent or a substitute for it was obtained from all the patients prior to their inclusion in the study.

Immunohistochemical analysis of IGFBP1 or IGFBP2. Immunohistochemical staining was performed using a peroxidase-labelled polymer conjugated to secondary antibodies (Histofine Simple Stain MAX PO (MULTI), Nichirei Co., Tokyo, Japan). Polyclonal rabbit antibodies against IGFBP1 (ab111203) and IGFBP2 (ab109284) were purchased from Abcam (Cambridge, UK). All the available H&E-stained slides of the surgical specimens were reviewed. For each case, representative paraffin blocks were selected for immunohistochemical studies. Four-micrometre-thick sections were cut from each formalin-fixed, paraffin-embedded tissue block. After deparaffinisation and rehydration, antigen retrieval treatment was administered at 400W (microwave) for 15 min in 10-mmoll sodium citrate buffer (pH 9.0), followed by treatment with 3% hydrogen peroxide for 15 min to quench the endogenous peroxidase activity.

The slides were incubated with the primary anti-IGFBP1 antibody (1:100) or anti-IGFBP2 antibody (1:100) overnight at 4˚C. Immunodetection was performed using the conventional streptavidin–biotin method with peroxidase-labelled SAB-PO kits (Nichirei Co, Tokyo, Japan). Diaminobenzidine substrate was used for colour development. The slides were counterstained with 1% Mayer's haematoxylin.

Interpretation of the immunostaining results. IGFBP1 and IGFBP2 expressions in the cytoplasm were evaluated using a scoring method based on the staining extent and staining intensity. The staining extent (positive frequency) was classified into four grades as per the percentage of stained tumour cells: 1 for <25%, 2 for 25% to <50%, 3 for 50% to <75% and 4 for ≥75% stained cells. The staining intensity was scored as per the following three grades: 0 (no staining [–] or weakly positive [±]), 1 (moderately positive [+]) and 2 (strongly positive [++]) (Figures 1 and 2). Composite scores were multiplied to produce a weighted score for each case (ranging from 1-6). For the statistical analyses, composite scores of ≥3 were defined as high expression, and scores <3 were considered to indicate low expression. Staining was assessed by three separate observers (YS, YT and MI) who were blinded to the patient data. Any disagreements among the three investigators were resolved by reassessment and consensus.

Table I. *Patient characteristics*.

	Entire cohort (n=219), n (%)
Gender	
Male	165 (75%)
Female	54 (25%)
Age (year) (median [range])	66.0 (21-92)
Main tumour location	
U	46 (21%)
ML	173 (79%)
Histopathology	
Differentiated	115 (52%)
Undifferentiated	104 (48%)
Depth of invasion	
T1	86 (39%)
T2	29 (13%)
T3	38 (17%)
T4	66 (30%)
Lymph node metastasis	
Positive	107 (49%)
Negative	112 (51%)
Stage classification	
Stage I	104 (48%)
Stage II	35 (16%)
Stage III	62 (28%)
Stage IV	18 (8%)

U: Upper; ML: middle or lower.

Statistical analyses. The χ^2 test was used to test the possible associations of IGFBP1 and IGFBP2 expression with clinicopathological variables. Kaplan–Meier curves were plotted to assess the effect of IGFBP1 and IGFBP2 expression on disease-specific survival (DSS). Different DSS curves were compared using the log-rank test. Multivariable proportional hazards Cox regression models were used to assess the prognostic significance of IGFBP1 and the factors associated with DSS. Any association with a p-value <0.10 on univariable analysis was included in the multivariable analysis. All p-values <0.05 were considered to indicate statistical significance. Statistical analyses were performed using IBM SPSS Statistics V.22 software (IBM, Armonk, NY, USA).

Results

Correlation between IGFBP1 protein expression and IGFBP2 protein expression. Among all patients, the numbers of patients with IGFBP1 high/IGFBP2 high, IGFBP1 high/IGFBP2 low, IGFBP1 low/IGFBP2 high and IGFBP1 low/IGFBP2 low were 41, 41, 25 and 112, respectively. IGFBP1 protein expression and IGFBP2 protein expression were positively correlated (p<0.001).

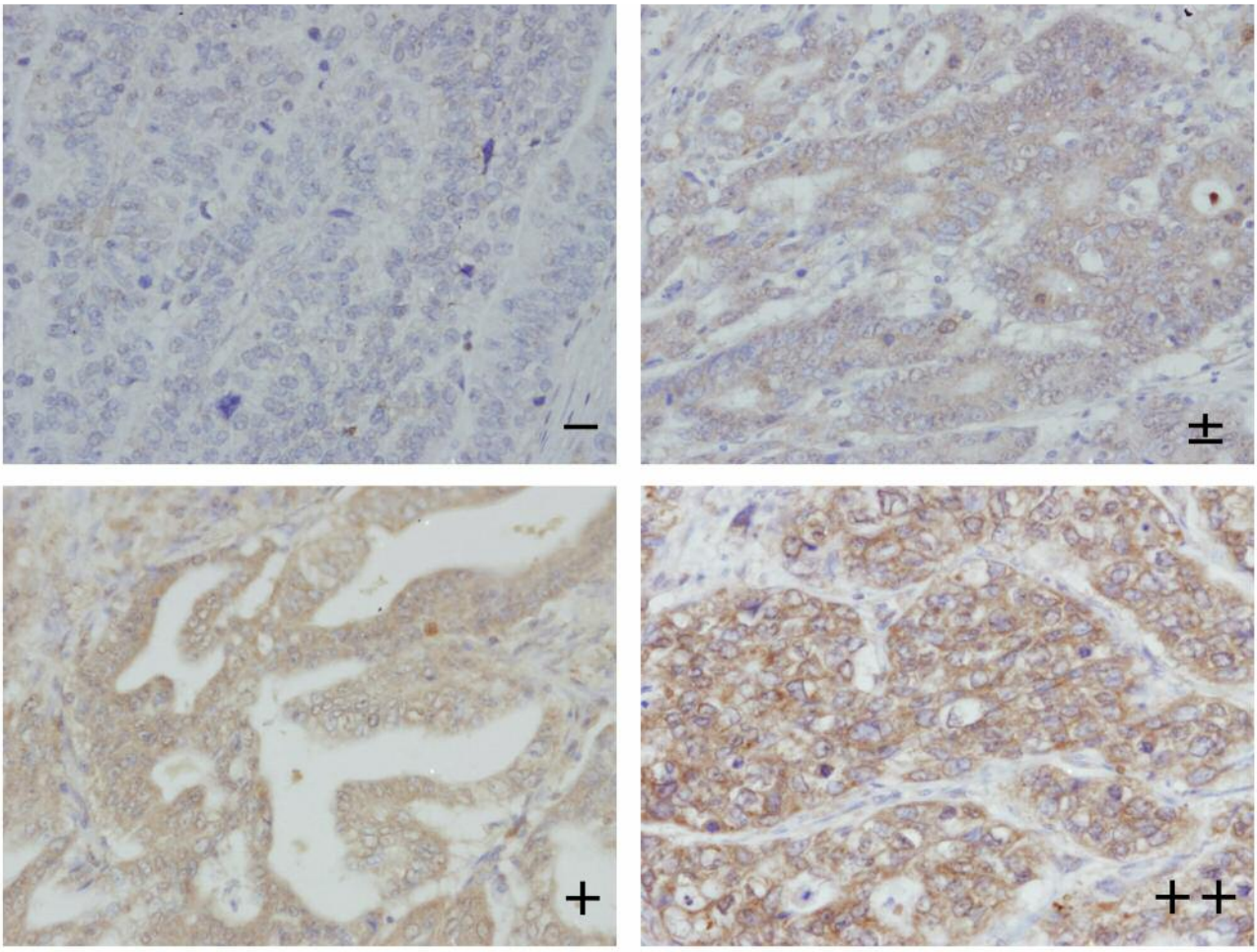

Figure 1. *Representative immunostaining of insulin-like growth factor binding protein-1 (IGFBP1) in gastric cancer. [–] No staining; [±] weakly positive; [+] moderately positive; [++] strongly positive.*

Correlation between IGFBP1/IGFBP2 protein expression and clinicopathological parameters. The correlation between IGFBP1/IGFBP2 protein expression and clinicopathological parameters is shown in Table II. The IGFBP1 expression was positively associated with the depth of invasion (T1 *vs*. T2-4, $p<0.001$), lymph node (LN) metastasis (N0 *vs*. N1-3, $p<0.001$), lymphatic invasion (negative *vs*. positive, $p<0.001$), venous invasion (negative *vs*. positive, $p<0.001$) and pathological stage (Stage I *vs*. II-IV, $p<0.001$).

IGFBP2 expression was associated with Lauren Classification (Intestinal *vs*. Diffuse, $p<0.001$) and was positively associated with the pathological stage (Stage I *vs*. II-IV, $p<0.001$).

Correlation between IGFBP1/IGFBP2 protein expression and DSS. The correlation of clinicopathological factors with DSS is shown in Table III. High IGFBP1 expression was significantly associated with a poorer DSS ($p<0.001$). The 5-year DSS of patients with high IGFBP1 expression was 59.1%, while that of those with low IGFBP1 expression was 80.0% (Figure 3A). The 5-year DSS of patients with high and low IGFBP2 was 71.5% and 73.0%, respectively, showing no significant difference ($p=0.667$) (Figure 3B). Tumour location (upper third of the stomach), Lauren Classification (diffuse type), depth of invasion (T2-4) and positive lymph node metastases were significantly associated with a poorer DSS in a univariable analysis. An adjusted multivariable analysis of the clinicopathological features affecting DSS indicated that IGFBP1 expression was not an independent prognostic factor (HR=1.2; 95%CI=0.74-2.1; $p=0.42$)] although depth of invasion and lymph node metastases were identified as independent prognostic factors (HR=10.7; 95%CI=2.5-45.8; $p=0.001$ and HR=6.0; 95%CI=2.5-13.4; $p<0.001$; respectively; Table II).

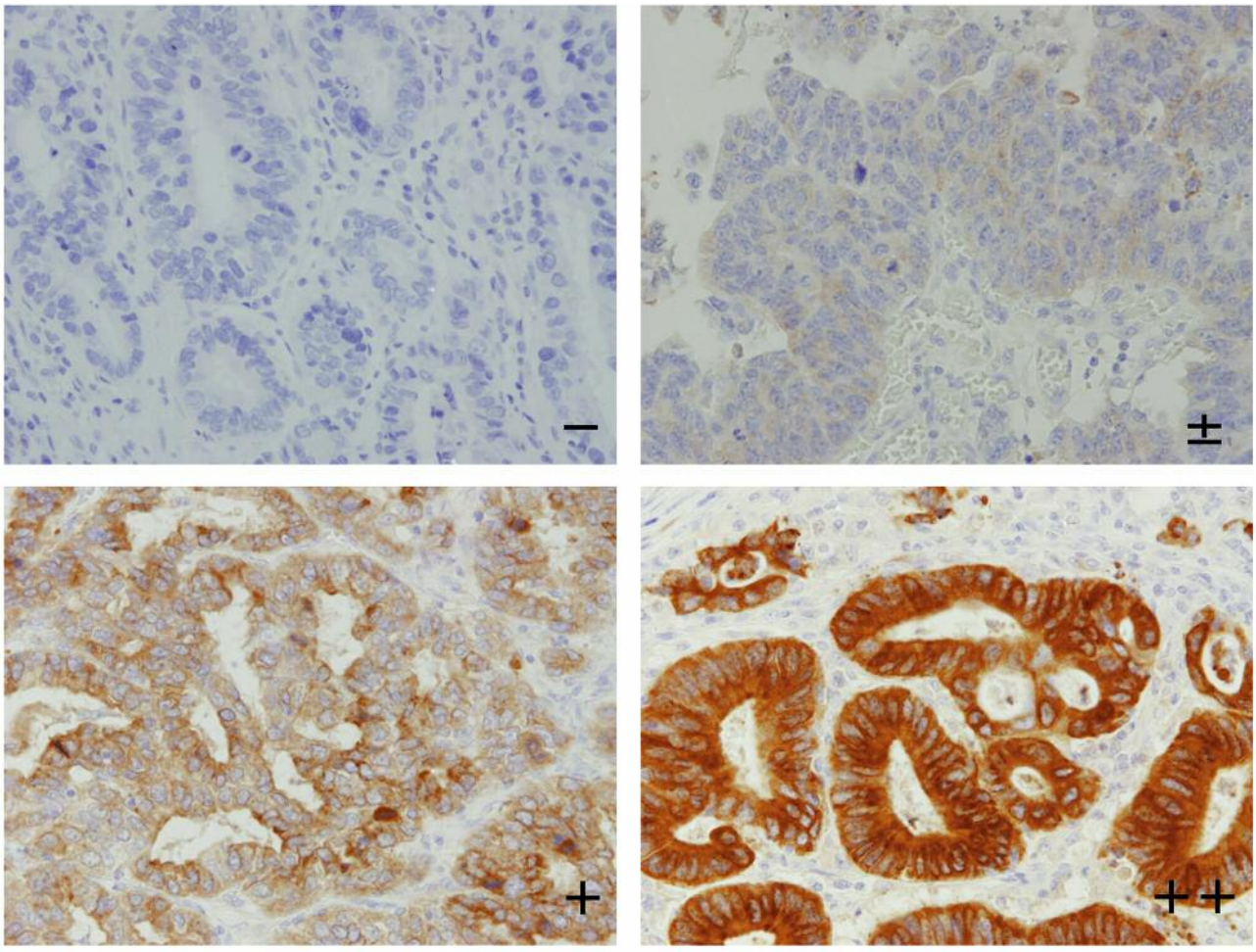

Figure 2. *Representative immunostaining of insulin-like growth factor binding protein-2 (IGFBP2) in gastric cancer. [–] No staining; [±] weakly positive; [+] moderately positive; [++] strongly positive.*

In a total of 217 patients who underwent radical gastrectomy without macroscopically residual tumour; high IGFBP1 expression was also significantly associated with a poorer RFS ($p<0.001$). The 5-year RFS of patients with high and low IGFBP1 expressions was 51.9% and 79.1%, respectively (Figure 4A). The 5-year RFS of patients with high and low IGFBP2 expressions was 69.9% and 68.9%, respectively ($p=0.925$) (Figure 4B).

Correlation between clinicopathogical factors and haematogenous metastasis. The correlation of the clinicopathogical factors and haematogenous metastasis is shown in Table IV. In univariate analysis, the depth of invasion (T2-4, $p=0.002$), LN metastasis (N1-3, $p=0.013$), lymphatic invasion (positive, $p=0.008$), pathological stage (Stage II-IV, $p=0.006$) and IGFBP1 expression (positive, $p<0.001$) were significantly correlated with haematogenous metastasis. Although IGFBP2 expression tended to be associated with haematogenous metastasis, no significant difference was seen between IGFBP2 high and low patients ($p=0.064$). In multivariate analysis, only IGFBP1 expression was found to be correlated with haematogenous metastasis.

Discussion

Our results showed that high IGFBP1 expression was significantly correlated with haematogenous metastasis and poor survival in patients with gastric cancer who underwent gastrectomy. To our knowledge, this is the first study that investigated the relationship of IGFBP1 expression with clinicopathological variables and prognosis in gastric cancer.

Few studies have evaluated the association of IGFBP1 expression with clinicopathological factors in solid cancer.

Table II. *Correlation between IGFBP1/IGFBP2 protein expression and clinicopathological parameters.*

	IGFBP1 expression				IGFBP2 expression			
	Low	High	Proportion	*p*-Value	Low	High	Proportion	*p*-Value
Age (years)								
≥65	67	49	42.2%	0.094	78	38	32.8%	0.381
<65	71	32	31.1%		75	28	27.2%	
Gender								
Male	99	66	40.0%	0.143	112	53	32.1%	0.308
Female	39	15	27.8%		41	13	24.1%	
Lauren Classification								
Intestinal	60	44	42.3%	0.126	61	43	41.3%	<0.001
Diffuse	78	37	32.2%		92	23	20.0%	
Depth of invasion								
T1	74	12	14.0%	<0.001	66	20	23.3%	0.097
T2, 3, 4	64	69	51.9%		87	46	34.6%	
LN metastasis								
Negative (N0)	84	28	25.0%	<0.001	83	29	25.9%	0.186
Positive (N1,2,3)	54	53	49.5%		70	37	34.6%	
Lymphatic invasion								
Negative	58	15	20.5%	<0.001	54	19	26.0%	0.355
Positive	80	66	45.2%		100	48	32.4%	
Venous invasion								
Negative	64	15	19.0%	<0.001	59	21	26.3%	0.363
Positive	74	66	47.1%		95	46	32.6%	
Stage								
I	83	21	20.2%	<0.001	80	24	23.1%	0.039
II/III/IV	55	60	52.2%		73	42	36.5%	

LN: Lymph node.

IGFBP1 expression has been reported to have positive or negative correlation with cancer risk in some types of cancer (13-15). Luo C *et al*. have investigated the expression pattern of IGFBP-1 of gastric adenocarcinoma infected with *H. pylori* and evaluated its role in the process of gastric cancer migration (16). The expression and release of IGFBP-1 were increased, with enhanced expression being associated with the migration ability of cancer cells in gastric cancer infected with *H. pylori*. They also reported that IGFBP-1 could modulate the MMP-9 expression and be involved in the process of *H. pylori*-induced MMP-9 expression, suggesting that IGFBP-1 may be a tumour-suppressor gene in the process of *H. pylori*-induced gastric cancer. However, this was an *in vitro* study using a cell line and limited to gastric cancer infected with *H. pylori*. Based on our results, IGFBP1 was suggested to be a tumour promoter that accelerated haematogenous metastasis and led to poor survival in gastric cancer. These findings suggest that IGFBP1 may potentially serve as a target of molecular targeted therapy in gastric cancer. In clinical practice, an anti-IGFBP1 drug may prevent haematogenous metastasis and improve survival in gastric cancer patients with high IGFBP1 protein expression.

We speculate that in gastric cancer patients, haematogenous metastasis may be promoted by the angiogenic action of IGFBP1 *via* both, IGF-dependent and IGF-independent pathways. *In vitro* studies have showed that IGFBP1 potentiates the action of IGFs, thereby increasing the migration of the endothelial cells in microvessels and large vessels (17, 18). Haematogenous metastasis may also be promoted by several IGF-independent pathways of IGFBP1 that reportedly accelerate angiogenesis (19-21).

In this study, IGFBP1 was a significant independent risk factor of haematogenous metastasis although IGFBP1 was not significantly correlated with DSS, based on multivariate analysis. This may be because haematogenous metastasis was not a major cause of cancer-related death. In this study, 68 patients died because of gastric cancer. Haematogenous metastasis was present only in 18 of these patients (15%). However, peritoneal metastasis was observed in 41 patients (65%) and was considered the main cause of cancer-caused death in this study.

Based on the present results, IGFBP2 was not significantly related to survival, although IGFBP2 is reported to have a positive relationship with clinicopathological factors in gastric cancer. Shi *et al*. have reported that the expression of IGFBP2 in gastric cancer was higher than that

Table III. *Prognostic factors for disease-specific survival (DSS) in univariate and multivariate Cox proportional-hazards regression models.*

	Univariate (Log-rank)		Multivariate (Cox regression)		
	5-years DSS	*p*-Value	HR	95%CI	*p*-Value
Age (years)					
≥65	68.2%	0.193			
<65	74.4%				
Gender					
Female	72.2%	0.907			
Male	70.7%				
Location					
ML	75.7%	0.004			
U	54.1%		1.53	0.91-2.56	0.10
Lauren Classification					
Intestinal	80.6%	0.005			
Diffuse	62.5%		1.43	0.84-2.42	0.19
Depth of invasion					
T1	97.7%	<0.001			
T2/3/4/	53.7%		10.73	2.51-45.82	0.001
LN metastasis					
Negative (N0)	94.6%	<0.001			
Positive (N1/2/3)	46.1%		5.95	2.46-13.37	<0.001
IGFBP1					
Low	80.0%	<0.001			
High	59.1%		1.23	0.74-2.05	0.42
IGFBP2					
Low	73.0%	0.667			
High	71.5%				

ML: Middle or lower; U: upper; LN: lymph node; DSS: disease-specific survival; HR: hazard ratio; CI: confidence interval.

in the normal gastric mucosa (9). Zhang *et al*. have reported a positive correlation between IGFBP2 expression and the depth of penetration, lymph node metastasis and clinical stage in gastric cancer (10). In some other cancers, it is thought that IGFBP2 promotes cancer progression (8). IGFBP2 induces cancer cell proliferation, survival and migration/invasion *via* mechanisms involving integrins and other pathways, including the Wnt pathway (22-24). In our study, IGFBP2 expression was more common in intestinal cancer and higher stage cancer and tended to be positively associated with haematogenous metastasis. However, there was no positive relationship between IGFBP2 expression and prognosis. Our sample size may have been insufficient to indicate a positive influence of IGFBP2 expression on prognosis. Our results indicate that the influence of IGFBP1 on prognosis will be more significant than that of IGFBP2.

This study has certain limitations. First, the mechanism by which IGFBP1 promoted haematogenous metastasis was not revealed. Further investigations, such as functional analyses using cell lines, are warranted to obtain an understanding of the biological function of IGFBP1 in gastric cancer. Second, the relation between the combination of expression of IGFBP subtypes and clinicopathological factors was not investigated. We speculate that IGFBP family members may interact with each other because IGF-independent actions of IGFBPs have also been described recently.

In conclusion, high IGFBP1 expression is associated with haematogenous metastasis and poor survival in patients with gastric cancer. IGFBP1 might become a new prognostic factor and a target of molecular targeted therapy for gastric cancer.

Conflicts of Interest

The Authors declare that they have no conflicts of interests.

Authors' Contributions

Study concepts: YS, MI; Study design: YS, MI, YT; Conducting the study: YS, MI, YT; Immunohistochemistry and analysis: YS, MI, YT; Data analysis and interpretation: YS, MI, YT; Statistical analysis: YS, MI; Manuscript preparation: YS, MI; Manuscript review and revise: YT, KK.

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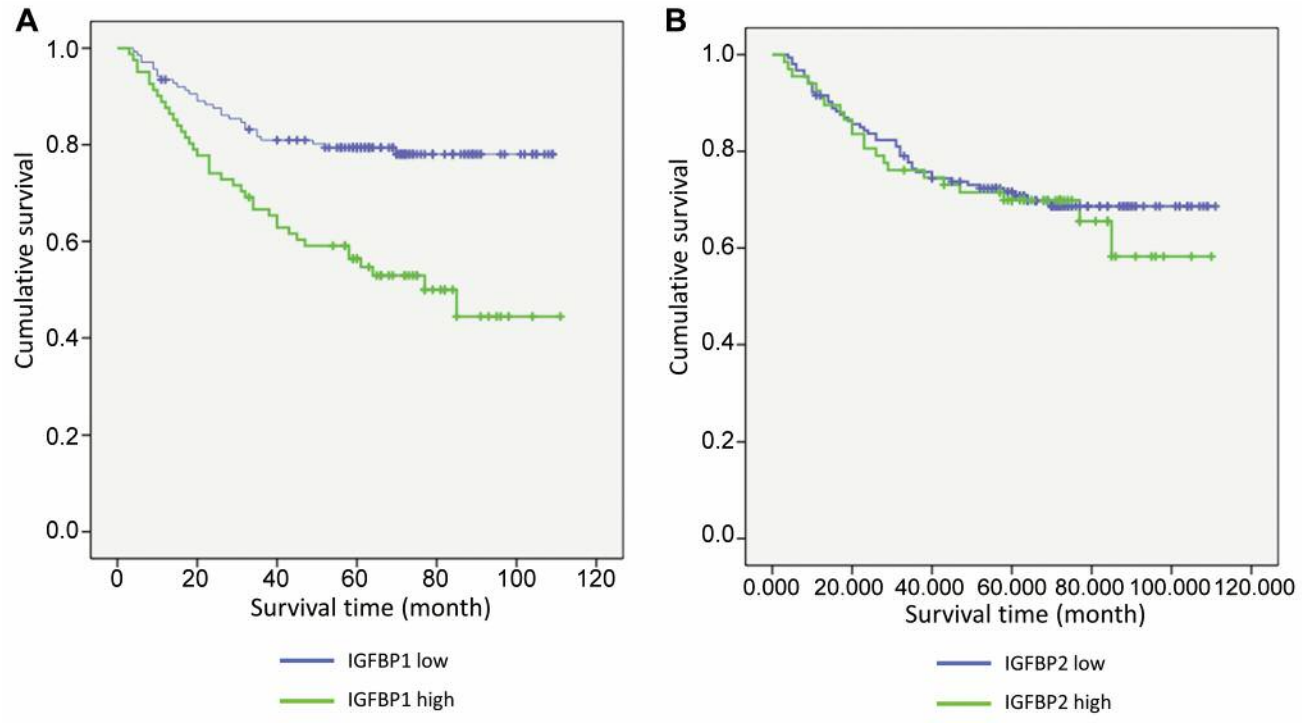

Figure 3. *Kaplan–Meier curves of the disease-specific survival (DSS) of patients with expression of IGFBP1 (A) or IGFBP2 (B).*

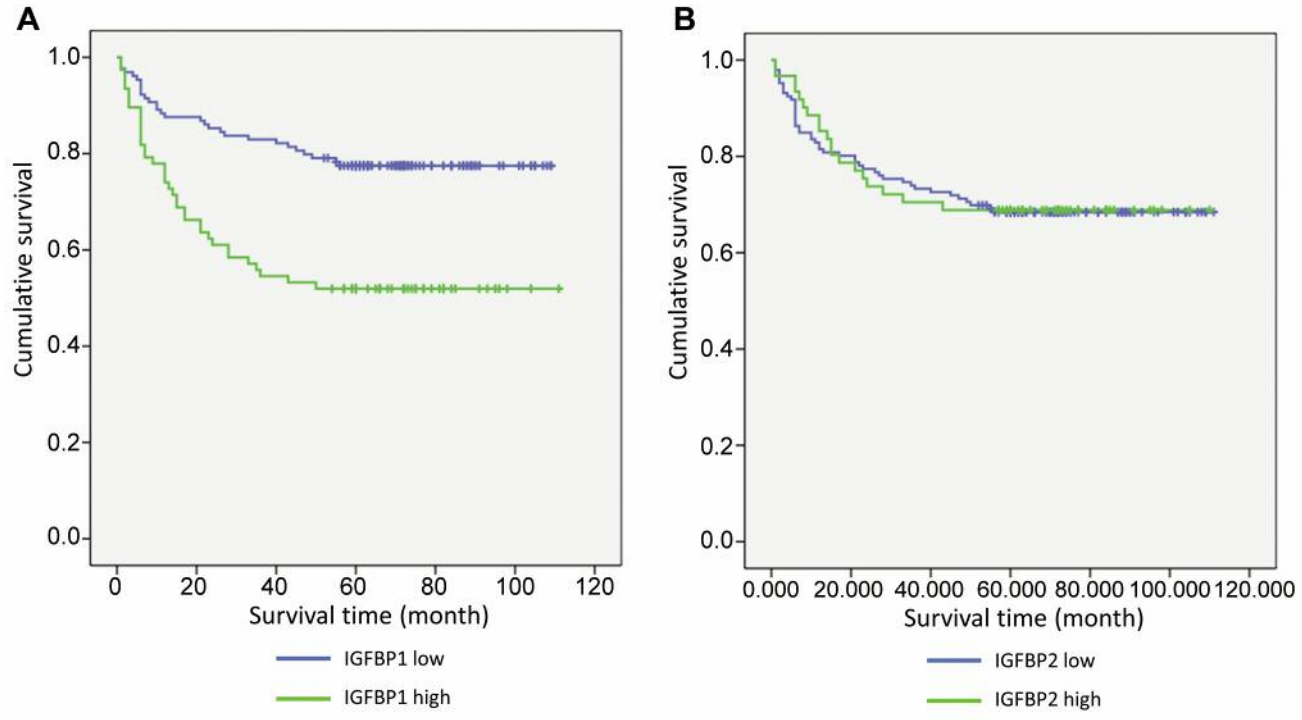

Figure 4. *Kaplan–Meier curves of the relapse-free survival (RFS) of patients with expression of IGFBP1 (A) or IGFBP2 (B).*

Table IV. *Correlation between clinicopathogical factors and haematogenous metastasis.*

	Haematogenous metastasis (–)	Haematogenous metastasis (+)	Proportion	*p*-Value	OR	95%CI	*p*-Value
Age (years)							
≥65	107	9	7.8%	0.81			
<65	94	9	8.7%				
Gender							
Male	148	17	10.3%	0.05	4.5	0.5-38.1	0.164
Female	53	1	1.9%				
Lauren Classification							
Intestinal	94	10	9.6%	0.62			
Diffuse	107	8	7.0%				
Depth of invasion							
T1	85	1	1.2%	0.002			
T1, T2, T3	116	17	12.8%		2.8	0.3-24.6	0.361
LN metastasis							
Negative (N0)	108	4	3.6%	0.013			
Positive (N1/2/3)	93	14	13.1%		1.2	0.3-5.0	0.282
Lymphatic invasion							
Negative	72	1	1.4%	0.008			
Positive	129	17	11.6%		3.6	0.3-39.4	0.33
Venous invasion							
Negative	76	3	3.8%	0.12			
Positive	125	15	10.7%				
Stage							
I	101	3	2.9%	0.006			
II/III/IV	100	15	13.0%				
IGFBP1 expression							
Low	137	1	0.7%	<0.001			
High	64	17	21.0%		22.7	2.7-188.0	0.004
IGFBP2 expression							
Low	144	9	5.9%	0.064			
High	57	9	13.6%		0.9	0.3-2.6	0.811

LN: Lymph node; OR: odds ratio; CI: confidential interval.

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