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Clinicopathological significance and impact on outcomes of the gene expression levels of *IGF*-1, *IGF*-2 and *IGF-1R*, *IGFBP*-3 in patients with colorectal cancer: Overexpression of the *IGFBP*-3 gene is an effective predictor of outcomes in patients with colorectal cancer

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Abstract. The insulin-like growth factors (IGF) system is involved in tumor proliferation, invasion and metastasis in cancer. The current study investigated the association of *IGF-1*, *IGF-2* and IGF-1 receptor (*IGF-1R*), IGF binding proteins type 3 (*IGFBP-3*) mRNA expression levels with clinicopathological characteristics and outcomes of 202 patients with untreated colorectal cancer (CRC). *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA expression levels were analyzed in surgical specimens of cancer tissues and adjacent normal mucosa cells using reverse transcription-quantitative polymerase chain reaction. The *IGF-1R* gene expression level was significantly higher in cancer tissue compared with adjacent normal mucosa. By contrast, *IGF-1* gene expression levels were reduced in cancer tissue compared with normal mucosa. *IGF-2* and *IGFBP-3* gene expression levels did not differ significantly between cancer tissue and adjacent normal mucosa. As for the association of gene expression and clinicopathological characteristics, *IGFBP-3* gene expression was significantly associated with lymph node metastasis. High *IGFBP-3* gene expression was associated with poor 5-year overall survival compared with patients with low *IGFBP-3* expression. Furthermore, *IGFBP-3* gene expression was identified as an independent prognostic factor using multivariate analysis. Overexpression of the *IGFBP-3* gene is considered an effective independent predictor of outcomes in patients with CRC.

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Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed types of cancer. Previous studies have suggested that an increased risk of CRC may be associated with dietary factors, blood insulin levels and the bioavailability of insulin-like growth factors (IGFs) (1-3).

The IGF system is a complex network consisting of two ligands (IGF-1 and IGF-2), two cell-surface receptors (IGF-1R and IGF-2R), a family of six high-affinity IGF-binding proteins (IGFBPs-1 to -6) and ≥4 additional low-affinity binding proteins (IGFBP-related proteins). This system is involved in normal cell growth, neoplastic transformation and tumor development. Imbalance of the IGF system has been implicated in the pathogenesis and progression of breast and other malignancies (3,4).

Abnormal expression of IGFs, as well as their receptors and binding proteins, has been identified in several malignancies, including CRC (5). IGF-1 may be able to increase the risk of cancer development (6). IGF-2 is also involved in tumor progression and patient survival and has been suggested to function as an autocrine growth factor in CRC (7). Overexpressed IGF-1R may promote invasion, tumor growth, metastasis and progression (8). Furthermore, ≥6 types of IGFBPs are expressed in most tissues and are present in the circulation in normal patients (9). These IGFBPs bind to IGFs with high affinity and the primary role of IGFBPs is to regulate the availability of IGFs for interactions with IGF-1R (10). From these, IGFBP-3 is the most abundant IGFBP in the circulation under normal circumstances, and has been focused on in numerous studies.

The association of the gene expression of the IGF system in tumors with the prognosis or clinicopathological characteristics of patients with CRC remains to be elucidated. In the present study, mRNA expression levels of the *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* genes were measured in cancer tissue and adjacent normal mucosa obtained from 202 patients with CRC. The focus of the current study was to evaluate the mRNA expression levels of the *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* genes and to determine whether expression levels are associated with clinicopathological characteristics and the clinical outcomes of patients with CRC.

Materials and methods

Patients and surgical specimens. A total of 202 patients with untreated CRC were enrolled into the present study. All patients underwent primary tumor resection at Gastroenterological Center at Yokohama City University Medical Center (Yokohama, Japan) or the Kanagawa Cancer Center (Kanagawa, Japan) between December 2002 and June 2006. Informed consent was obtained from each patient and the Ethical Review Boards at Yokohama City University and Kanagawa Cancer Center approved the present study. None of the patients had received chemotherapy or radiotherapy prior to surgery or had any other malignancies. Tumor staging was evaluated according to the 7th edition of the International Union Against Cancer Tumor-Node-Metastasis classification of malignant tumors (11). The resected tumor and adjacent normal mucosa were obtained from the resected colorectum, embedded in Tissue Tek OCT medium (Sakura Finetek Europe B.V., Felmingweg, Netherlands), frozen in liquid nitrogen and stored at -80˚C until used for RNA extraction. Sections of 5-μm thickness were stained with hematoxylin and eosin, and histopathological features were examined using a light microscope (CH30; Olympus Corporation, Tokyo, Japan). Sections that consisted of >80% carcinoma cells were defined as cancer tissue and used for total RNA extraction. The clinicopathological characteristics of the patients with CRC are presented in Table I.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA in resected CRC and adjacent normal mucosa was isolated with the use of TRIzol® Reagent (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). A total of 10 units of DNase I, RNase-free (Roche Applied Science, Penzberg, Germany) was added and the samples were incubated for 20 min at 37˚C. Complementary (c)DNA was synthesized from 0.2 μg of total RNA using the iScript cDNA Synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Reverse transcription was performed in a total volume of 20 μl, which contained 4 μl iScript reaction mix, 1 μl iScript reverse transcriptase, and 15 μl (13.3 ng/μl) total RNA. The complete reaction mix was incubated for 5 min at 25˚C, 30 min at 42˚C, 5 min at 85˚C. Following synthesis, the cDNA was diluted to 0.2 μg/μl with H_2O and stored at -20˚C until required for experiments.

RT-qPCR was performed with an iQ SYBR-Green Supermix kit (Bio-Rad Laboratories, Inc.). PCR reactions were performed in a total volume of 15 μl, which contained cDNA derived from 75 ng of RNA, 0.27 μM of each primer, 7.5 μl of iQ SYBR-Green Supermix containing dATP, dCTP, dGTP, and dTTP (400 μM each) and iTag DNA polymerase (50 units/ml). The PCR consisted of 10 min at 95˚C, followed by 40 cycles of denaturation of the cDNA for 10 sec at 95˚C, annealing for 10 sec at an appropriate temperature (Table II) and a primer extension for 20 sec at 72˚C, followed by 10 min at 72˚C. To distinguish specific from nonspecific products and primer dimmers, melting curve analysis was performed. RT-qPCR experiments were performed in triplicate, with two wells for each gene in each experiment. To evaluate specific mRNA expression in samples, a standard curve was produced for each run, measuring three points of the human control cDNA (Clontech Laboratories, Inc., Mountain View, CA, USA). The concentration of each sample was calculated by relating its crossing point to the standard curve (12).

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics 20 (IBM SPSS, Armonk, NY, USA). The gene expression levels in cancer tissue were compared with those in adjacent normal mucosa using the Wilcoxon signed-rank test. The associations between gene expression and potential explanatory variables (including age, gender, tumor location, tumor size, histological type, depth of invasion, lymph node metastasis, lymphatic invasion, venous invasion and liver metastasis) were evaluated using the χ^2 test. The gene expression levels in the tumors were compared in the presence or absence of lymph node metastasis. Kaplan-Meier curves for the postoperative survival of patients with CRC were plotted and differences in survival rate between groups were analyzed according to the log-rank test. A Cox proportional-hazards model was used to estimate the hazard ratios of variables for postoperative survival. Univariate and multivariate analyses were conducted using a Cox proportional-hazards model to identify independent prognostic factors for postoperative survival. Variables that had a P-value of <0.05 for at least one endpoint on univariate analysis were subsequently included in multivariate analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of the IGF-1, IGF-2, IGF-1R and IGFBP-3 genes in cancer tissue and adjacent normal mucosa. *IGF-1* gene expression levels were significantly reduced in cancer tissue compared with adjacent normal mucosa (P<0.001; Fig. 1A). *IGF-2* gene expression levels did not differ significantly between cancer tissue and adjacent normal mucosa (P=0.453; Fig. 1B). *IGF-1R* gene expression levels were significantly higher in cancer tissue compared with adjacent normal mucosa (P<0.001; Fig. 1C). *IGFBP-3* gene expression levels did not differ significantly between cancer tissue and adjacent normal mucosa (P=0.126; Fig. 1D).

Association of IGF-1, IGF-2, IGF-1R and IGFBP-3 mRNA expression levels to clinicopathological characteristics. Expression levels of the *IGF-1, IGF-2, IGF-1R* and *IGFBP-3* genes were categorized as low or high according to the median values (0.129, 0.362, 0.306 and 292.5, respectively). The associations between gene expression and clinicopathological characteristics were evaluated. Expression levels of

Table I. Associations between the intratumoral expression levels of IGF-1, IGF-2, IGF-1R and IGFBP-3 genes and the clinicopathological characteristics of patients with colorectal cancer.

Characteristics	IGF-1 expression			IGF-2 expression			IGF-1R expression			IGFBP-3 expression		
	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value
Age, years												
<60	28	28	1.000	27	29	0.753	30	26	0.530	28	28	1.000
≥60	73	73		74	72		71	75		73	73	
Gender												
Male	52	58	0.397	51	59	0.258	50	60	0.158	56	54	0.778
Female	49	43		50	42		51	41		45	47	
Tumor location												
Colon	62	48	0.048	62	48	0.048	54	56	0.778	53	57	0.572
Rectum	39	53		39	53		47	45		48	44	
Tumor diameter, cm												
≤5	69	62	0.302	73	58	0.027	66	65	0.883	71	60	0.105
>5	32	39		28	43		35	36		30	41	
Histological type												
Well differentiated	30	29	0.987	30	29	0.668	27	32	0.605	29	30	0.668
Moderately differentiated	57	58		55	60		58	57		60	55	
Poorly differentiated	14	14		16	12		16	12		12	16	
Depth of invasion												
T1	9	8	0.734	9	8	0.781	10	7	0.232	13	4	0.072
T2	19	14		19	14		19	14		18	15	
T3	36	42		38	40		32	46		39	39	
T4	37	37		35	39		40	34		31	43	
Lymph node metastasis												
Absent	53	50	0.673	52	51	0.888	48	55	0.325	59	44	0.035
Present	48	51		49	50		53	56		42	57	
Lymphatic invasion												
Absent	64	68	0.554	64	68	0.554	62	70	0.237	61	71	0.139
Present	37	33		37	33		39	31		40	30	
Venous invasion												
Absent	41	34	0.308	44	31	0.058	31	44	0.058	43	62	0.109
Present	60	67		57	70		70	57		58	69	

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Table I. Continued.

Characteristics	IGF-1 expression			IGF-2 expression			IGF-1R expression			IGFBP-3 expression		
	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value	Low n=101	High n=101	P-value
Liver metastasis												
Absent	83	77	0.298	82	78	0.488	80	80	1.000	84	76	0.165
Present	18	24		19	23		21	21		17	25	

IGF, insulin-like growth factor; IGF-1R, IGF receptor type 1; IGFBP-3, IGF binding protein type 3.

the *IGFBP-3* gene were significantly associated with lymph node metastasis (P=0.035; Table I). The *IGF-1* and *IGF-2* gene expression levels were significantly associated with tumor location (P=0.048, P=0.048, respectively).

Association of IGF-1, IGF-2, IGF-1R and IGFBP-3 mRNA expression levels and lymph node metastasis. No significant associations were identified between *IGF-1*, *IGF-2* and *IGF-1R* mRNA expression levels and lymph node metastasis (Fig. 2A-C). *IGFBP-3* mRNA expression levels were significantly increased in patients with lymph node metastasis (P=0.028; Fig. 2D).

Association between IGF-1, IGF-2, IGF-1R and IGFBP-3 mRNA expression levels and postoperative survival rate. The expression levels of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA were categorized as low or high according to the respective median values. Post-operative survival did not differ significantly according to expression levels of the *IGF-1*, *IGF-2* or *IGF-1R* genes (Fig. 3A-C). By contrast, postoperative survival was significantly poorer in patients with high expression levels of the *IGFBP-3* gene compared with those with low expression levels (Fig. 3D; P=0.003). Univariate analysis revealed that the depth of invasion, lymph node metastasis, lymphatic invasion, liver metastasis, tumor diameter and *IGFBP-3* expression were associated with clinical outcomes (P<0.01). On multivariate Cox proportional-hazards regression analysis, lymph node metastasis, liver metastasis and *IGFBP-3* gene expression were independently inversely correlated with patient outcomes (P=0.011, P<0.001, P=0.026, respectively; Table III).

Discussion

The IGF system serves an important role in the pathogenesis of dysplasia and neoplasia (8,13). The present study investigated tissue expression levels of the *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* genes, the clinicopathological characteristics of 202 patients with untreated CRC, and the associations of these expression levels with postoperative survival.

A number of previous studies have compared the mRNA expression levels of the *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* genes in CRC tissue and adjacent normal mucosa. Freier *et al* (14) reported that there was no identifiable IGF-1 mRNA in healthy or malignant human colonic tissue. Nosho *et al* (15) reported significantly increased mRNA expression levels of the *IGF-2* gene in colorectal tumor tissue compared with those in adjacent normal mucosa. The mRNA expression levels of the *IGF-1R* gene were higher in adenocarcinoma tissue of the colon compared with adjacent normal mucosa (14). Another previous study demonstrated that mRNA expression of the *IGF-1R* gene was detected in ~40% of CRC tissue specimens but was undetectable in adjacent normal mucosa (15). Keku *et al* (16) reported that *IGFBP-3* gene expression was significantly lower in colorectal adenoma tissue compared with normal mucosa. In the current study, the mRNA expression level of the *IGF-1R* gene was higher in CRC tissue compared with adjacent normal mucosa. However, the mRNA expression levels of the *IGF-1* gene were reduced in cancer tissue compared with adjacent normal mucosa. The

Table II. Primers and conditions for the polymerase chain reaction.

Gene/internal control	Primers	Probes (5'-3')	Annealing temperature (˚C)	Product size (bp)
IGF-1	Forward	GTGGATGAGTGCTGCTTC	58.0	134
	Reverse	ACTTCCTTCTGGGTCTTGG		
IGF-2	Forward	TACCGCCATCTCCCTTCTC	60.0	122
	Reverse	TCCCTCTGACTGCTCTGTG		
IGF-1R	Forward	TGCCTTGGTCTCCTTGTC	58.0	154
	Reverse	TTTCCCTGCTTTGATGGTC		
IGFBP-3	Forward	TTTCATCTCTCATCTTTTGTCCTC	60.0	77
	Reverse	GCCATTCCTCCTTCCTGTTC		
β-actin	Forward	AGTTGCGTTACACCCTTTCTTGAC	60.0	171
	Reverse	GCTCGCTCCAACCGACTGC		

IGF, insulin-like growth factor; IGF-1R, IGF-receptor 1; IGFBP, IGF-binding protein; bp, base pair.

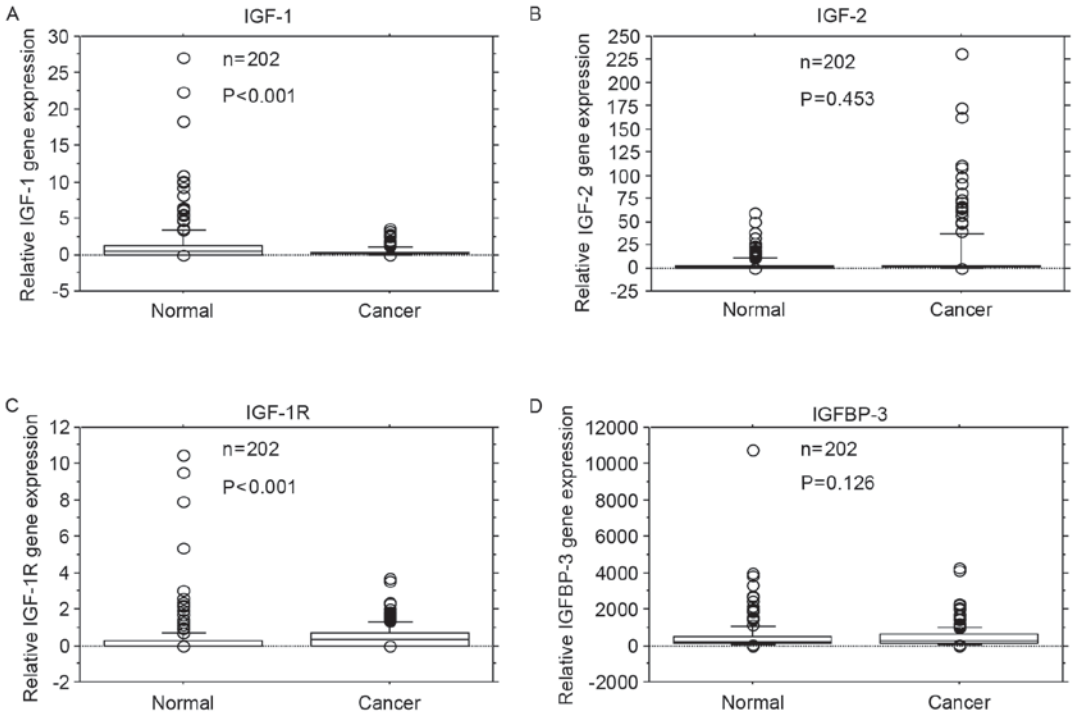

Figure 1. Comparison of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA expression levels in colorectal cancer tissue (Cancer) and adjacent normal mucosa (Normal). (A) *IGF-1* gene expression levels were lower in cancer tissue compared with adjacent normal mucosa (P<0.001). (B) *IGF-2* gene expression levels did not differ significantly between cancer tissue and adjacent normal mucosa (P=0.453). (C) *IGF-1R* gene expression levels were significantly higher in cancer tissue compared with adjacent normal mucosa (P<0.001). (D) *IGFBP-3* gene expression levels did not differ significantly between cancer tissue and adjacent normal mucosa (P=0.126). Box boundaries demonstrate the 25th and 75th percentiles of the observed values. Capped bars indicate the 10th and 90th percentiles and the solid line presents the median average. The P-values were calculated with the Wilcoxon signed-rank test. IGF, insulin-like growth factors; IGF-1R, IGF-1 receptor; IGFBP, IGF binding protein.

mRNA expression levels of the *IGF-2* and *IGFBP-3* genes did not differ significantly between cancer tissue and adjacent normal mucosa.

The association between *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA expression levels and clinicopathological characteristics were examined in patients with CRC. Peters *et al* (17) reported that *IGF-1* gene expression was not associated with any clinicopathological characteristic in CRC, whilst Shiratsuchi *et al* (18) reported that IGF-1 gene expression in CRC was associated with tumor size, depth of tumor invasion, lymphatic invasion and venous invasion in CRC. In another previous study, *IGF-2* gene expression was correlated with age and tumor size, whilst *IGF-1R* gene expression did not associate with any clinicopathological characteristic in patients with early CRC (15). Although *IGF-1R* expression correlated with tumor size and depth of invasion in CRC (18), it did not associate any of the clinicopathological characteristics in patients with prostate cancer (19). Increased postoperative tumor growth and the presence of liver metastasis were associated with significantly elevated *IGF-1R* gene expression in

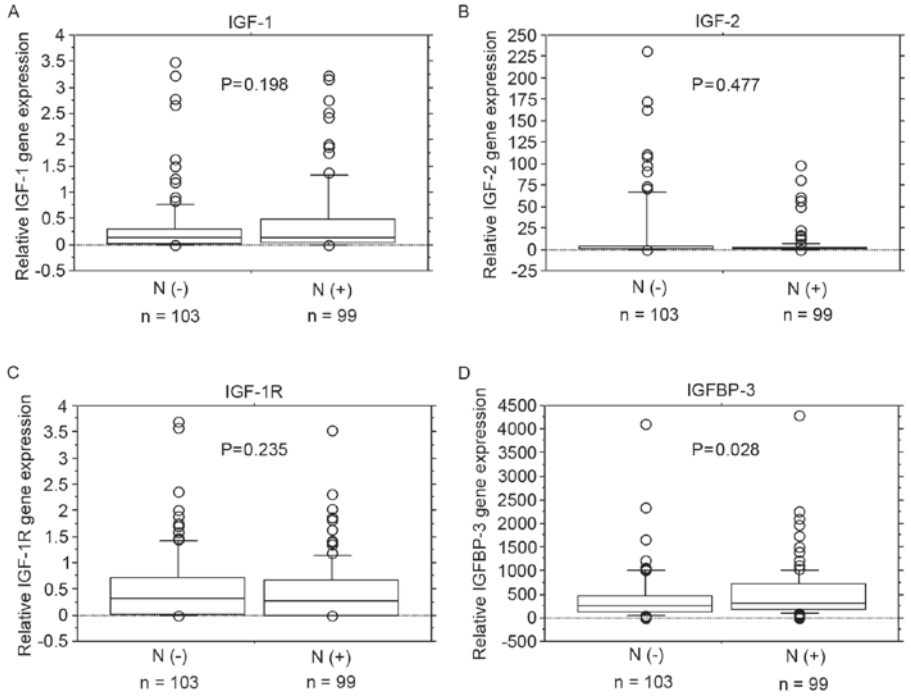

Figure 2. mRNA expression levels of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* in lymph node metastasis. No significant associations were identified between (A) *IGF-1*, (B) *IGF-2* or (C) *IGF-1R* mRNA expression levels and lymph node metastasis. (D) *IGFBP-3* mRNA expression levels were significantly higher in patients with lymph node metastasis (P=0.028). Box boundaries demonstrate the 25th and 75th percentiles of the observed values. Capped bars indicate the 10th and 90th percentiles and the solid line presents the median average. The P-values were calculated with the Mann-Whitney U test. IGF, insulin-like growth factors; IGF-1R, IGF-1 receptor; IGFBP, IGF binding protein; N(-), negative for lymph node metastasis; N(+), positive for lymph node metastasis.

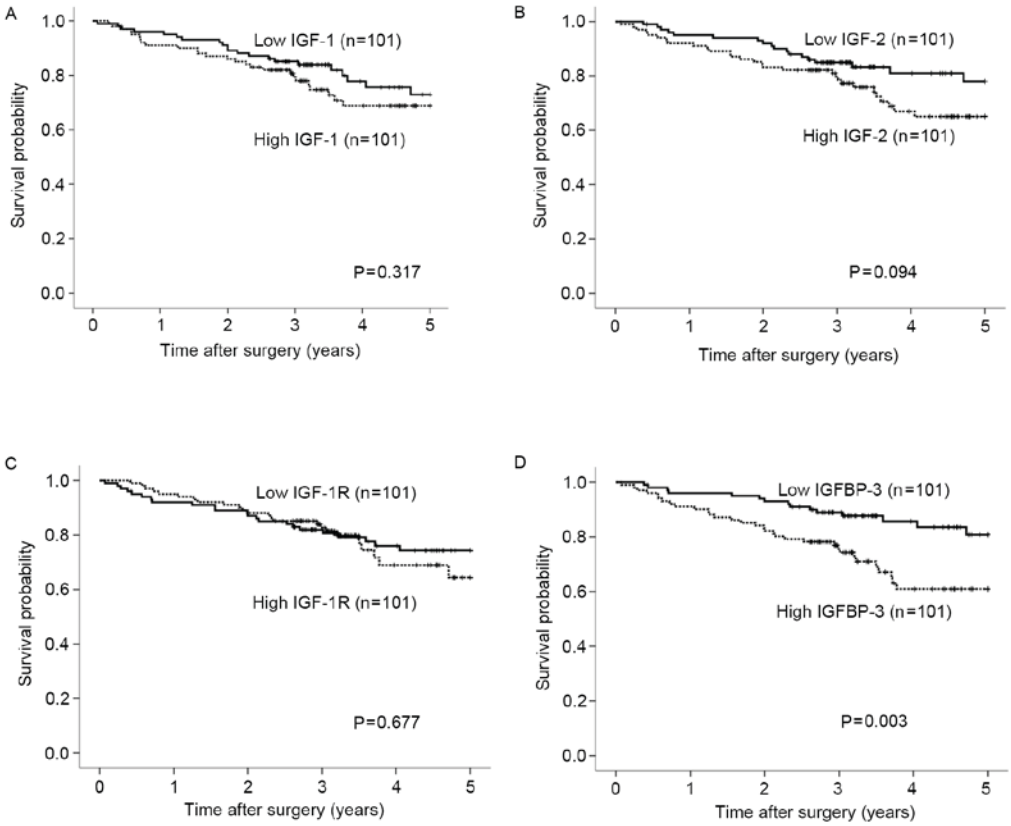

Figure 3. Association of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA expression levels with postoperative survival rate. Postoperative survival did not differ significantly according to expression levels of the (A) *IGF-1*, (B) *IGF-2* or (C) *IGF-1R* genes. (D) By contrast, postoperative survival rate was significantly poorer in patients with high expression levels of the I*GFBP-3* gene compared with those with low expression levels (P=0.003). Expression levels of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* mRNA were categorized as low or high according to the respective median values. The postoperative survival rate was analyzed using the Kaplan-Meier method and differences in survival rates were assessed with the use of the log-rank test. Results have a median follow-up of 3.24 years. IGF, insulin-like growth factors; IGF-1R, IGF-1 receptor; IGFBP, IGF binding protein.

Table III. Univariate and multivariate analyses using the Cox proportional hazard model of variables associated with the postoperative survival of patients with colorectal cancer.

	Univariate analysis			Multivariate analysis		
Variables	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Gender						
Male vs. female	1.330	0.742-2.385	0.338	-	-	-
Age, years						
≥60 vs. <60	1.367	0.695-2.688	0.365	-	-	-
Depth of invasion						
T3/4 vs. T1/2	17.687	2.439-128.27	0.004	5.650	0.746-42.81	0.094
Lymph node metastasis						
Present vs. absent	6.383	2.979-13.676	<0.001	3.038	1.292-7.144	0.011
Tumor location						
Rectum vs. colon	1.513	0.851-2.699	0.158	-	-	-
Lymphatic invasion						
Present vs. absent	3.307	1.849-5.912	<0.001	1.604	0.834-3.085	0.157
Venous invasion						
Present vs. absent	1.601	0.827-3.099	0.163	-	-	-
Liver metastasis						
Present vs. absent	7.258	4.033-13.063	<0.001	4.695	2.511-8.780	<0.001
Histological type						
Moderate and poor vs. well	2.045	0.955-4.380	0.066	-	-	-
Tumor diameter, cm						
≤5 vs. >5	2.191	1.236-3.884	0.007	1.225	0.677-2.215	0.502
IGF-1 expression level						
High vs. low	1.340	0.754-2.382	0.319	-	-	-
IGF-2 expression level						
High vs. low	1.645	0.914-2.914	0.097	-	-	-
IGF-1R expression level						
High vs. low	1.130	0.635-2.011	0.677	-	-	-
IGFBP-3 expression level						
High vs. low	2.439	1.320-4.507	0.004	2.033	1.087-3.804	0.026

IGF-1, insulin-like growth factor type 1; IGF-1R, IGF receptor type 1; IGFBP-3, IGF binding protein type 3; CI, confidence interval.

gastrinoma (20). In the present study, *IGF-1* gene expression was significantly associated with tumor location. *IGF-2* gene expression was significantly associated with tumor location and tumor size; whilst *IGF-1R* gene expression was not associated with any clinicopathological characteristic in patients with CRC. In a previous study, *IGFBP-3* gene expression was significantly associated with age and positively correlated with tumor stage in CRC (21). Higher mRNA levels of the *IGFBP-3* gene were associated with reduced levels of apoptosis (16). Positive associations of IGFBP-3 expression with tumor size and lymph node metastasis have been identified in oral squamous cancer (22). An increased expression level of IGFBP-3 has been associated with lymph node metastasis in pancreatic endocrine neoplasms (23). The *IGFBP-3* gene was overexpressed in advanced pancreatic cancer and the intratumoral levels of the *IGFBP-3* gene was associated with tumor size (24). The results of the current study are in accordance with the literature that *IGFBP-3* gene overexpression is associated with lymph node metastasis.

Finally, the association of *IGF-1*, *IGF-2*, *IGF-1R* and *IGFBP-3* gene expression levels and the outcomes of patients with CRC were assessed. In previous studies, IGF-1 expression was not observed to be significantly associated with overall survival rate (17,25). IGF-2 expression has been associated with poorer clinical outcomes in patients with CRC (7,17,26). IGF-1R expression in primary CRC may promote an increased risk of recurrence (27), but was not associated with patients' 5-year survival rate (28). Increased tissue expression levels of the *IGFBP-3* gene have been associated with the rapid growth of breast cancer and poor patient outcomes (29,30). Previous immunohistochemical studies of breast cancer demonstrated that IGFBP-3 expression was associated with shorter overall

survival (31). High expression levels of the *IGFBP-3* gene were associated with unfavorable prognostic characteristics in breast cancer (32). Santosh *et al* (33) reported that IGFBP-3 overexpression in tumor tissues was an independent predictor of reduced survival rate in patients with newly diagnosed glioblastoma. By contrast, Aishima *et al* (34) identified that high expression levels of IGFBP-3 were independently associated with an improved survival rate in patients with hepatocellular carcinoma. In patients with squamous cell carcinoma of the tongue, IGFBP-3 expression was associated with favorable outcomes (35). In the present study, the postoperative survival rate was significantly poorer in patients with high expression levels of the *IGFBP-3* gene compared with those with low expression levels of the *IGFBP-3* gene.

The molecular mechanisms underlying the association between IGFBP-3 expression and poor outcomes in cancer remain to be fully elucidated. Schmid *et al* (36) reported that IGFBP-3 was overexpressed in the endothelial cells of mouse breast tumor vessels. Granata *et al* (37) reported that IGFBP-3 dose-dependently promoted neovessels in subcutaneous implants *in vivo* and suggested that IGFBP-3 promotes angiogenesis and positively regulates the expression of proangiogenic molecules, including vascular endothelial growth factor (VEGF) (37). Yu *et al* (38) reported a positive correlation between the high expression of epidermal growth factor receptor (EGFR) and the high expression of IGFBP-3 in breast cancer tissue, and Butt *et al* (10) identified that a blockade of EGFR kinase activity restored the inhibitory effects of IGFBP-3 *in vitro*. Additionally, Martin *et al* (39) demonstrated that IGFBP-3 enhanced EGF signaling and its proliferative effects via increased EGFR phosphorylation and the activation of MAP kinase signaling pathways in breast cancer cells *in vitro*. An associated previous study also reported that IGFBP-3 promotes the proliferation of breast cancer cells through increasing EGFR signaling mediated by SphK1 (40). Therefore, IGFBP-3 has been suggested to promote angiogenesis by inducing VEGF, thereby inducing EGFR signaling mediated by SphK1 and activation of MAP kinase signaling pathways. These effects are considered to promote proliferation of cancer cells, potentially leading to poor survival outcomes. Molecular investigations are required to additionally investigate the role of IGFBP-3 as a prognostic factor and to elucidate the pleiotropic functions of this protein.

In conclusion, high expression of the *IGFBP-3* gene was significantly associated with lymph node metastasis and poor outcomes. The results of the present study suggest that overexpression of the *IGFBP-3* gene is an important independent predictor of outcomes following surgery in patients with CRC.

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