Am J Cancer Res 2025;15(3):1174-1188
www.ajcr.us /ISSN:2156-6976/ajcr0163525

Original Article

Direct triangular comparison of tissue and serum growth differentiation factor 15 with host factors in colorectal cancer

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Received January 23, 2025; Accepted March 7, 2025; Epub March 15, 2025; Published March 30, 2025

Abstract: Growth differentiation factor 15 (GDF-15) is a potential biomarker for colorectal cancer (CRC) and is associated with sarcopenia and cachexia. However, its clinical significance in CRC remains unclear. We investigated the clinical significance of GDF-15 in CRC patients by a unique triangular comparison of tissue and preoperative serum GDF-15 levels with host factors. We evaluated 428 tissue and 214 serum samples from 214 CRC patients. We measured tissue and serum levels of GDF-15 and assessed their association with oncological outcomes and host factors. While cancer tissue GDF-15 levels showed no significant associations with clinicopathological factors or survival, preoperative serum GDF-15 levels were significantly correlated with indicators of disease progression, such as advanced T stage and advanced pathological stage. High preoperative serum GDF-15 level was associated with poor disease-free survival and overall survival and was an independent prognostic factor for disease-free survival and overall survival. Significant correlations were observed between preoperative serum GDF-15 levels and host factors, including body mass index, psoas muscle mass index, intramuscular adipose tissue content, and C-reactive protein. In conclusion, preoperative serum GDF-15 reflects host factors such as body composition and inflammation and is a useful marker for the oncological management of CRC patients.

Keywords: Cachexia, colorectal cancer, growth differentiation factor 15, prognostic biomarker, sarcopenia

Introduction

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor beta superfamily [1]. GDF-15 levels increase in response to a variety of stress signals, such as inflammation and hypoxia, and GDF-15 has been associated with a range of diseases, including atrial fibrillation, heart failure, renal failure, and cancer [2-4]. The biological significance of GDF-15 in cancer has long been studied, and recent research has elucidated multiple signaling pathways through which GDF-15 exerts its effects (**Figure 1A**). One key pathway is the GDF-15-GFRAL axis, which has been reported to contribute to poor prognosis in cancer patients by inducing sarcopenia and cachexia through appetite suppression and metabolic alterations [5-9]. Additionally, GDF-15 has been suggested to promote Nrf2 activation, thereby protecting cancer cells from oxidative stress and facilitating the development of chemoresistance [10]. Furthermore, GDF-15 has been shown to enhance cancer metastasis by activating the epithelial-mesenchymal transition via the TGF-β signaling pathway [11]. Collectively, these pathways highlight the complex and multifaceted role of GDF-15 in cancer progression and host metabolic responses, underscoring its significance in tumor biology. In a recent clinical trial, ponsegromab, a humanized monoclonal antibody inhibiting GDF-15, increased weight gain and overall activity level and reduced cachexia symptoms in patients with cancer cachexia and elevated GDF-15 levels, including in patients with colorectal cancer

https://doi.org/10.62347/WTCF3616

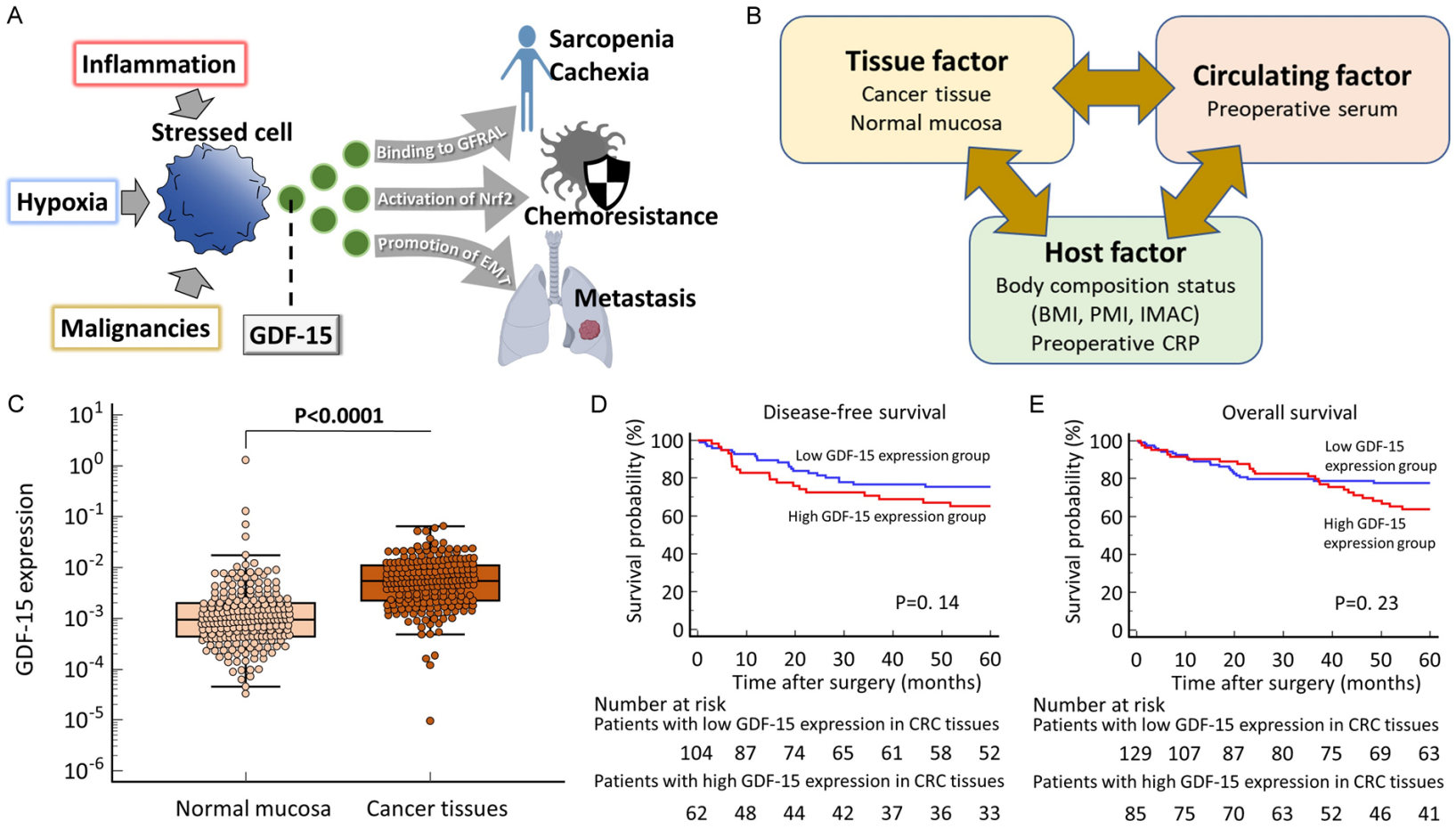


Figure 1. A. A schematic overview of the role of GDF-15. B. A schematic overview of the study. C. Scattered boxplot of GDF-15 expression levels in CRC tissues and adjacent normal mucosa. D, E. Kaplan-Meier analysis of DFS and OS of CRC patients stratified by GDF-15 expression levels in CRC tissues. The number at risk, shown below the time axis, indicates the number of patients remaining in follow-up without an event at each time point. CRC, colorectal cancer; DFS, disease-free survival; OS, overall survival; GDF-15, growth differentiation factor 15.

(CRC) [12]. Furthermore, an ongoing clinical trial co-administering the GDF-15-blocking antibody visugromab with the anti-PD-1 antibody nivolumab suggests that neutralizing GDF-15 may potentially overcome resistance to immune checkpoint inhibitors in solid cancers [13]. Further understanding of the clinical value of GDF-15 in cancer is needed.

Cancer cachexia is a multiorgan syndrome marked by severe malnutrition accompanied with loss of skeletal muscle, which negatively impacts patient quality of life and survival [14]. Myopenia, a component of cachexia, is a reduction in skeletal muscle mass, independent of factors such as illness or aging [15]. Research has revealed the clinical burden of myopenia and its impact on prognosis across various cancer types, including CRC, particularly in the preoperative setting [16, 17]. Several lines of evidence have suggested the close relationship between sarcopenia or cachexia with the systemic inflammatory response (SIR) via tumor-host interactions [16, 18]. The Global Leadership Initiative on Malnutrition, which established a global consensus on the identification and endorsement of malnutrition diagnostic criteria, considers SIR as one of the etiologic factors for malnutrition [19]. Moreover, the Asian Working Group for Cachexia recently developed a consensus on diagnostic criteria for cachexia in Asia; these criteria also include SIR, such as an elevated C-reactive protein level above 0.5 mg/dL, as one of the diagnostic criteria for cachexia [20]. While studies have shown the upregulation of GDF-15 in response to stress signals including inflammation, the correlation between tissue or serum GDF-15 levels in patients with malignancies, including CRC, with SIR has not been examined. Moreover, most previous studies focused solely on tissue or serum GDF-15 levels in relation to oncological outcomes. No studies have examined the relationship between GDF-15 levels and host factors, such as inflammation, nutritional status, and skeletal muscle mass, in patients with malignancies.

In this study, we systematically analyzed tissue and preoperative serum GDF-15 levels in CRC patients and conducted a unique approach involving a direct triangular comparison with various preoperative host factors to determine the clinical significance of GDF-15 in CRC (**Figure 1B**).

Material and methods

Patients and tissue samples

We enrolled 214 patients with CRC who underwent surgical treatment at our institution between January 2011 and December 2015. Patients who were treated with radiotherapy or chemotherapy before surgery were excluded from this study. Surgical approaches included laparotomy and laparoscopy with standard curative resection. CRC tissues and adjacent normal mucosa from all patients were preserved immediately after surgical resection in RNAlater (Qiagen, Hilden, Germany) and stored at -80°C until RNA extraction. Tissue samples were subjected to histopathological analysis and classified following the Union for International Cancer Control (UICC) tumor node metastasis (TNM) staging system. All patients with stage III/IV CRC received 5-fluorouracil-based chemotherapy, whereas those with stage I or II CRC received no adjuvant chemotherapy. Patients were observed at 3-month intervals for 2 years after surgery, at 6-month intervals for the subsequent 3 years, and annually thereafter. A medical history was obtained and physical examination was conducted at each visit; chest X-ray, colonoscopy, and computed tomography were performed each year. Data collected from inpatient and outpatient records included the following: demographic data (age and sex); tumor-specific details (histology, location, T classification, venous and lymphatic invasion, lymph node metastasis, distant metastasis); and survival data (disease-free survival (DFS) and overall survival (OS)). All participants provided written informed consent. The study protocol was approved by the Institutional Review Board of Mie University. This study was performed in accordance with The Declaration of Helsinki.

Quantitative reverse transcription-PCR (qRT-PCR)

The surgical specimens were homogenized using a Mixer Mill MM 300 homogenizer (Qiagen, Chatsworth, CA, USA). Total RNA was isolated using a RNeasy Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. cDNA was synthesized with random hexamer primers and Superscript III reverse transcriptase (Thermo Fisher Scientific,

Waltham, MA, USA) following the manufacturer's instructions. Target gene expression was determined by qRT-PCR using Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) and the StepOnePlus Real Time PCR System (Applied Biosystems). The primers for GDF-15 were as follows: forward, GAGCTGGGAAGATTCGAACA and reverse, AGAGATACGCAGGTGCAGGTG. GAPDH mRNA served as the internal control, and the primer sequences were as follows: forward, GGAAGGTGAAGGTCGGAGTC and reverse, AATGAAGGGGTCATTGATGG. PCR amplification was conducted under the following conditions: 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min. Target gene expression was determined using the standard curve method, and GDF-15 mRNA expression levels were normalized using GAPDH mRNA expression levels.

Detection of serum CRP and GDF-15 levels

Serum samples collected within 1 week before surgery from all patients were used for analysis, and CRP levels were measured in routine blood tests. Serum GDF-15 levels were analyzed using the human GDF-15 immunoassay Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol. Briefly, serum samples were diluted fourfold, and diluted samples were added to a microplate pre-coated with a monoclonal antibody specific for human GDF-15. The plate was incubated for 2 h at room temperature on a horizontal orbital shaker. Each well was then aspirated and washed four times with wash buffer to ensure the removal of any unbound substances. Human GDF-15 conjugate was added to each well, followed by a 1-h incubation at room temperature on the shaker. Wells were aspirated and washed again as described. Substrate solution was added to each well, and the plate was incubated for 30 min at room temperature, protected from light. Stop solution was added to stop the reaction. The optical density was measured at 450 nm immediately after stopping the reaction, with correction at 570 nm. The results were calculated using a standard curve generated from a four-parameter logistic curve and expressed in ng/ml. Measurements were performed in duplicate, and mean values were calculated.

Image analysis

Preoperative CT scans were assessed within 4 weeks before surgery. Preoperative CT scan images were stored in an electronic format suitable for image analysis to assess body composition status. Using preoperative plain CT at the superior aspect of the fourth lumbar vertebra as previously described [17, 21], we measured the cross-sectional area of the bilateral psoas muscles by manual tracing. We calculated the psoas muscle mass index (PMI) as follows: PMI = cross-sectional area of bilateral psoas muscle/height^2 (cm^2/m^2). Low PMI was regarded as a proxy for low muscle volume, as previously described [22-24]. The cross-sectional area of the subfascial muscular tissue in the multifidus muscle was traced manually at the same level on the plain CT image, and mean CT values (Hounsfield units (HU)) for these areas were determined using the Aquarius NET server (TeraRecon Inc., San Mateo, CA, USA). We also measured CT values (HU) at the region of interest (ROI) in the subcutaneous fat by placing four circles on areas of subcutaneous fat located away from major vessels at the same level, as described previously [25]. Intramuscular adipose tissue content (IMAC) was calculated using the ratio of the CT values as follows: IMAC = mean CT value of the ROI of the multifidus muscle (HU)/mean CT value of the ROI of the subcutaneous fat (HU). High IMAC is considered a proxy for low muscle quality [26].

Statistical methods

Statistical analysis was performed using MedCalc version 22.023 (Mariakerke, Belgium). Categorical variables were compared using the chi-square test. Non-parametric data comparisons between two independent groups were performed using the Mann-Whitney U test. Correlations between continuous variables were evaluated using Spearman's rank correlation coefficient. Receiver operating characteristic curves with Youden's index were generated to determine the optimal cutoff values of GDF-15, PMI, and IMAC for DFS. The cutoff values for PMI and IMAC were determined separately for each sex, as previously described [17, 26]. The optimal cutoff values were 6.554 cm^2/m^2 for PMI in males, 4.592 cm^2/m^2 for PMI in females, -0.452 for IMAC in males, and -0.353 for IMAC in females. The cutoffs for BMI

and CRP were set at 21 kg/m^2 and 0.5 mg/dL (5.0 mg/L), respectively, as reported in a previous publication [20]. For time-to-event analyses, survival estimates were calculated using Kaplan-Meier analysis, and groups were compared with the log-rank test. DFS was measured from the date the patient underwent curative surgery to the date of disease recurrence, death from any cause (i.e., cancer-unrelated deaths were not censored), or until the last contact with the patient. OS was measured from the date the patient underwent surgery until the date of death from any cause (i.e., cancer-unrelated deaths were not censored) or the last known follow-up for patients who were still alive. Cox proportional hazards models were used to estimate hazard ratios (HRs) for recurrence or death. We used multivariate logistic regression models to predict the factors influencing preoperative serum GDF-15 levels. Variables with a *P*-value <0.05 in univariate analysis were selected for the multivariate analysis using a Cox proportional hazards model or a logistic regression model. All *p* values were two-sided, and P<0.05 was considered statistically significant.

Results

Overexpression of the GDF-15 gene in CRC tissues

The patient population comprised 123 men and 91 women, median age 66 years, range 31-94 years. Forty-eight (22.4%) patients had stage I CRC, 58 (27.2%) had stage II, 60 (28.0%) had stage III, and 48 (22.4%) had stage IV. The median follow-up time was 58 months (range: 1-147 months). In clinical samples from patients with CRC, quantitative real-time RT-PCR analysis showed that GDF-15 levels were significantly elevated in cancerous tissues compared with adjacent normal mucosa (P<0.0001, **Figure 1C**).

Clinical significance of GDF-15 expression in CRC tissues

We evaluated the association between GDF-15 levels in cancerous tissues in CRC patients and clinicopathological factors; no statistically significant associations were identified (**Table 1A**). To evaluate the association between GDF-15 levels in cancerous tissues and prognosis in CRC patients, Kaplan-Meier survival curves stratified by GDF-15 levels were generated through time-to-event analysis. No significant correlation was observed between GDF-15 expression levels in CRC tissues and DFS and OS (log-rank test, P=0.14, P=0.23, respectively; **Figure 1D** and **1E**).

Increased preoperative serum GDF-15 levels were significantly associated with CRC development

Next, we performed an analysis of preoperative serum GDF-15 levels. Stratification of patients into high and low preoperative serum GDF-15 groups revealed a trend toward higher GDF-15 expression in CRC tissues among patients with elevated serum GDF-15 levels; however, this difference did not reach statistical significance (P=0.06, **Figure 2A**). We further evaluated the association between preoperative serum GDF-15 levels and clinicopathological factors in CRC patients. Significant associations were found between high preoperative serum GDF-15 levels and several clinicopathological factors indicative of disease progression, including advanced pathological T stage (P=0.02), presence of distant metastasis (P=0.03), and advanced pathological stage classification (P= 0.01) (**Table 1B**). A significant association was also observed with older age (> median, P<0.0001).

Elevated preoperative serum GDF-15 levels were significantly associated with poor oncological outcome in CRC

To assess the potential of preoperative serum GDF-15 as a prognostic biomarker, we next conducted Kaplan-Meier survival curve analysis using preoperative serum GDF-15 levels to perform time-to-event analysis. Patients with increased preoperative serum GDF-15 levels had significantly poorer prognosis in terms of DFS (log-rank test, P=0.02; **Figure 2B**) and OS (log-rank test, P<0.0001; **Figure 2C**). In Cox univariate proportional hazards analysis, male sex, left side tumor, advanced T classification (T3/T4), lymphatic vessel invasion, lymph node metastasis, and high preoperative serum GDF-15 levels were associated with poor DFS (**Table 2A**). In multivariate analysis, high preoperative serum GDF-15 level was identified as an independent prognostic factor for DFS (HR: 1.98, 95% CI: 1.09-3.59, P=0.02; **Table 2A**); left side tumor and lymph node metastasis were also

Table 1A. Clinicopathological variables by level of GDF-15 expression in CRC tissues

Variables		N	GDF-15 expression in CRC tissues		*P* value
			High† (N=85)	Low† (N=129)	
Sex	Male	123	51	72	0.55‡
	Female	91	34	57	
Median age (years)	>66	108	43	65	0.98‡
	≤66	106	42	64	
Histological type	Differentiated	195	79	116	0.45‡
	Undifferentiated	19	6	13	
Location	Right	65	21	44	0.14‡
	Left	149	64	85	
Pathological T category	pT1/T2	61	19	42	0.11‡
	pT3/T4	153	66	87	
Venous invasion	Present	141	57	84	0.77‡
	Absent	73	28	45	
Lymphatic invasion	Present	131	51	80	0.77‡
	Absent	83	34	49	
Lymph node metastasis	Present	97	41	56	0.49‡
	Absent	117	44	73	
Distant metastasis	Present	48	23	25	0.19‡
	Absent	166	62	104	
Pathologic stage	Stage I	48	15	33	0.41‡
	Stage II	58	24	34	
	Stage III	60	23	37	
	Stage IV	48	23	25	

CRC: colorectal cancer; GDF-15: growth differentiation factor 15. †Cutoff thresholds for levels of GDF-15 expression in CRC tissues were determined by receiver operating characteristic curve analysis with Youden's index for disease-free survival in CRC patients. ‡Chi-square test.

identified as independent prognostic factors. Univariate analysis showed that undifferentiated histology, advanced T classification (T3/T4), venous invasion, lymph node metastasis, distant metastasis, and high preoperative serum GDF-15 levels were associated with poor OS (**Table 2B**). Multivariate Cox regression analysis revealed that high preoperative serum GDF-15 level was an independent prognostic factor for poor OS in CRC patients (HR: 2.47, 95% CI: 1.46-4.18, P=0.0008) (**Table 2B**).

There was a significant association between preoperative serum GDF-15 levels and body composition status and inflammation in CRC

We next examined the relationship between GDF-15 and body composition status and inflammation. No significant correlation was found between GDF-15 expression in cancer tissue and preoperative BMI (Rho=-0.02, P=0.81, **Figure 3A**), PMI (Rho=-0.09, P=0.21, **Figure 3B**), IMAC (Rho=-0.02, P=0.77, **Figure 3C**), or preoperative serum CRP levels (Rho=0.05, P=0.47, **Figure 3D**). Stratification by low and high preoperative BMI (P=0.42, **Figure 3E**), PMI (P=0.42, **Figure 3F**), IMAC (P=0.45, **Figure 3G**), or preoperative serum CRP levels (P=0.32, **Figure 3H**) did not reveal any significant differences in GDF-15 expression in cancerous tissues. In contrast, preoperative serum GDF-15 levels showed a significant negative correlation with BMI (Rho=-0.15, P=0.03, **Figure 3I**) and preoperative PMI (Rho=-0.26, P=0.0001, **Figure 3J**). A strong positive correlation was confirmed between preoperative serum GDF-15 levels and IMAC (Rho=0.16, P=0.02, **Figure 3K**) and preoperative serum CRP levels (Rho=0.42, P<0.0001, **Figure 3L**). Furthermore, stratification by low and high preoperative BMI, PMI, IMAC, and serum CRP levels showed a clear association between elevated preoperative serum GDF-15 levels and the low BMI group (P=0.008, **Figure 3M**), low PMI group (P<

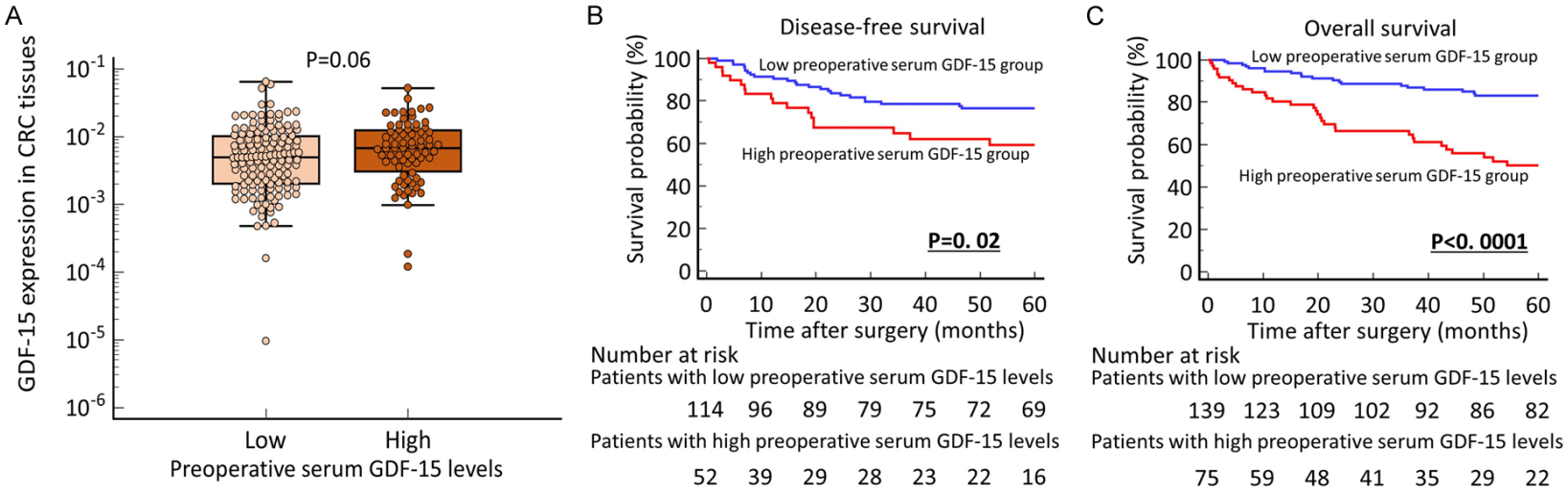


Figure 2. A. Scattered boxplots of GDF-15 expression in cancer tissues stratified by high and low preoperative serum GDF-15 levels. B, C. Kaplan-Meier analysis of DFS and OS of CRC patients stratified by preoperative serum GDF-15 levels. The number at risk, shown below the time axis, indicates the number of patients remaining in follow-up without an event at each time point. CRC, colorectal cancer; DFS, disease-free survival; OS, overall survival; GDF-15, growth differentiation factor 15.

Table 1B. Clinicopathological variables by level of preoperative serum GDF-15 levels in CRC patients

Variables		N	Preoperative serum GDF-15 levels		*P* value
			High† (N=75)	Low† (N=139)	
Sex	Male	123	49	74	0.09‡
	Female	91	26	65	
Median age (years)	>66	108	53	55	<0.0001*,‡
	≤66	106	22	84	
Histological type	Differentiated	195	66	129	0.24‡
	Undifferentiated	19	9	10	
Location	Right	65	25	40	0.49‡
	Left	149	50	99	
Pathological T category	pT1/T2	61	14	47	0.02*,‡
	pT3/T4	153	61	92	
Venous invasion	Present	141	50	91	0.86‡
	Absent	73	25	48	
Lymphatic invasion	Present	131	44	87	0.58‡
	Absent	83	31	52	
Lymph node metastasis	Present	97	34	63	1.0‡
	Absent	117	41	76	
Distant metastasis	Present	48	23	25	0.03*,‡
	Absent	166	52	114	
Pathologic stage	Stage I	48	10	38	0.01*,‡
	Stage II	58	25	33	
	Stage III	60	17	43	
	Stage IV	48	23	25	

*P<0.05. CRC: colorectal cancer; GDF-15: growth differentiation factor 15. †Cutoff thresholds for preoperative serum GDF-15 levels were determined by receiver operating characteristic curve analysis with Youden's index for disease-free survival in CRC patients. ‡Chi-square test.

Table 2A. Multivariate analysis for predictors of disease-free survival in CRC patients

Variables	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender (Male)	1.99	1.08-3.66	0.03*	1.45	0.77-2.73	0.25
Age (>66 years†)	1.14	0.65-2.01	0.65			
Histological type (Undifferentiated)	1.10	0.34-3.54	0.87			
Location (Left)	4.09	1.74-9.62	0.001*	5.17	2.13-12.57	0.0003*
T classification (pT3/4)	2.69	1.34-5.39	0.005*	2.19	0.99-4.84	0.05
Venous invasion (Present)	1.72	0.94-3.16	0.08			
Lymphatic invasion (Present)	1.81	1.0-3.27	0.048*	1.06	0.53-2.12	0.88
Lymph node metastasis (Present)	2.65	1.51-4.67	0.0007*	2.82	1.52-5.26	0.001*
GDF-15 expression in CRC tissues (High‡)	1.53	0.87-2.68	0.14			
Preoperative serum GDF-15 levels (High‡)	2.0	1.13-3.54	0.02*	1.98	1.09-3.59	0.02*

*P<0.05. CI: confidence interval; CRC: colorectal cancer; GDF-15: growth differentiation factor 15; HR: hazard ratio. †The median age at surgery was 66 years in this cohort. ‡Cutoff thresholds for tissue GDF-15 expression levels and preoperative serum GDF-15 levels were individually determined by receiver operating characteristic curve analysis using Youden's index for of disease-free survival in CRC patients.

0.0001, **Figure 3N**), high IMAC group (P=0.03, **Figure 3O**), and high CRP group (P<0.0001, **Figure 3P**).

Finally, we investigated risk factors for preoperative elevated serum GDF-15 in CRC patients. Univariate analysis demonstrated that

Table 2B. Multivariate analysis for predictors of overall survival in CRC patients

Variables	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Gender (Male)	1.40	0.83-2.35	0.21			
Age (>66 years†)	0.97	0.59-1.60	0.92			
Histological type (Undifferentiated)	2.78	1.41-5.49	0.003*	1.92	0.96-3.85	0.07
Location (Left)	1.61	0.89-2.92	0.12			
T classification (pT3/4)	2.36	1.23-4.54	0.01*	0.93	0.44-1.97	0.85
Venous invasion (Present)	2.10	1.16-3.82	0.01*	1.16	0.60-2.22	0.66
Lymphatic invasion (Present)	1.54	0.91-2.61	0.11			
Lymph node metastasis (Present)	2.47	1.47-4.17	0.0007*	1.26	0.68-2.34	0.46
Distant metastasis (Present)	8.54	5.05-14.47	<0.0001*	6.62	3.45-12.69	<0.0001*
GDF-15 expression in CRC tissues (High‡)	1.35	0.82-2.23	0.24			
Preoperative serum GDF-15 levels (High‡)	2.91	1.76-4.81	<0.0001*	2.47	1.46-4.18	0.0008*

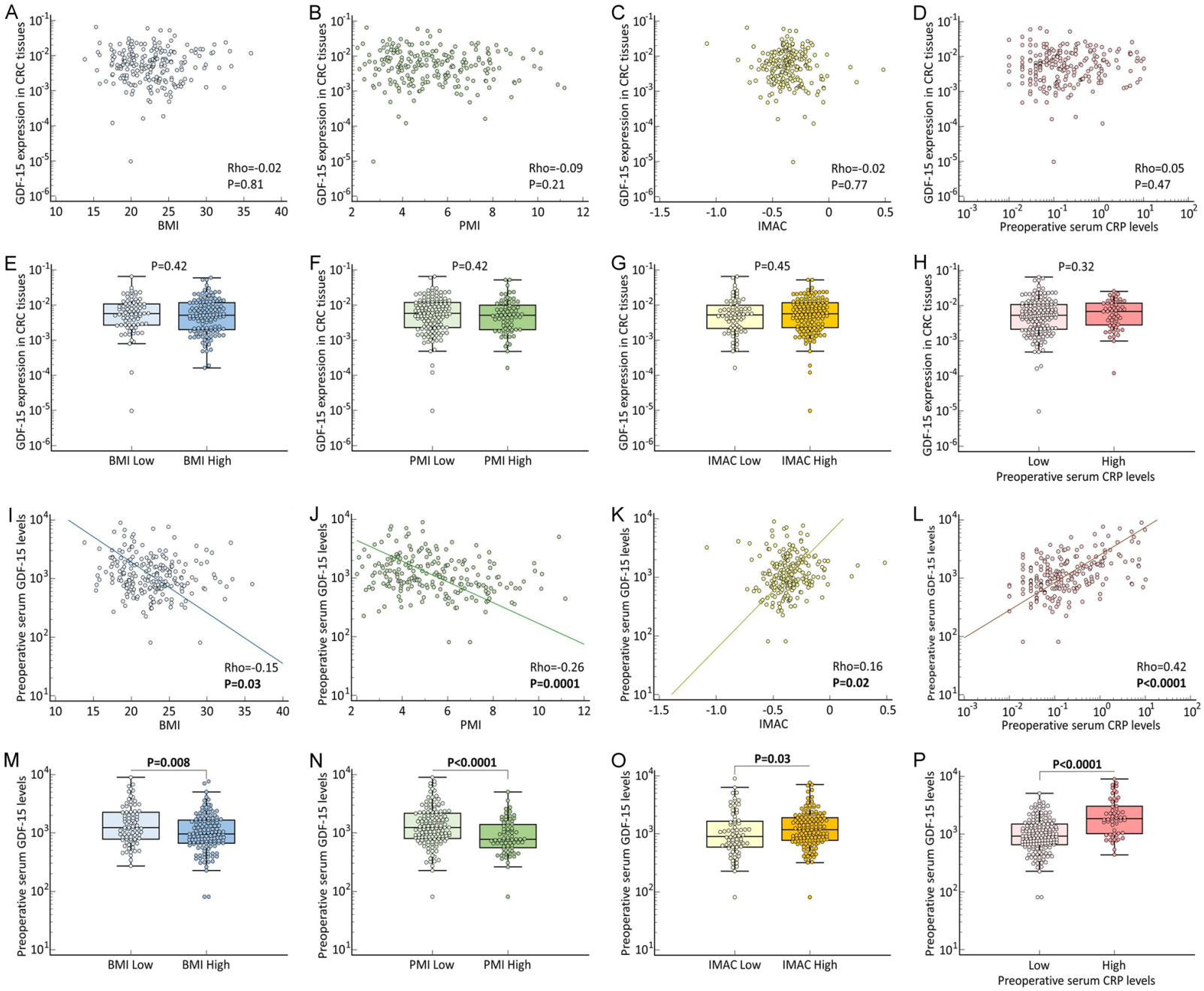
*P<0.05. CI: confidence interval; CRC: colorectal cancer; GDF-15: growth differentiation factor 15; HR: hazard ratio. †The median age at surgery was 66 years in this cohort. ‡Cutoff thresholds for tissue GDF-15 expression levels and preoperative serum GDF-15 levels were individually determined by receiver operating characteristic curve analysis using Youden's index for disease-free survival in CRC patients.

older age (P<0.0001), advanced T classification (P=0.02), presence of distant metastasis (P=0.04), decreased preoperative BMI (P= 0.04), decreased preoperative PMI (P=0.004), and increased preoperative CRP level (P< 0.0001) were significantly associated with elevated serum GDF-15 concentration. Multivariate logistic regression analysis identified older age (OR: 5.08, 95% CI: 2.40-10.74) (P<0.0001) and high preoperative serum CRP levels (OR: 7.69, 95% CI: 3.44-17.18) (P<0.0001) as independent risk factors for elevated preoperative serum GDF-15 levels (**Table 3**).

Discussion

GDF-15, also known as macrophage inhibitory cytokine 1 (MIC-1), placental transforming growth factor-beta (pTGFB), and placental bone morphogenetic protein (PLAB), was first reported as a novel member of the TGF-β superfamily [1]. While initial studies focused on the association between GDF-15 and stress responses such as inflammation [27], recent studies have revealed various functions of GDF-15 in cancer. Some reports have shown that GDF-15 exhibits tumor-suppressive functions by inducing cell growth arrest and apoptosis, particularly in the initial stages of tumorigenesis [28-30]. Other studies have suggested an oncogenic role of GDF-15 in processes such as epithelial-to-mesenchymal transition, angiogenesis, metastasis, the acquisition of drug and radiation resistance, and immune evasion [31-36]. The underlying mechanisms regarding its role in these processes remain incompletely understood.

Increasing studies have demonstrated diverse roles of GDF-15, particularly in various diseases, including malignancies [37, 38]. Johnen et al. reported that GDF-15 plays a role in tumor-induced anorexia and weight loss [6]. Recent findings have demonstrated that GDF-15 mediates anorexia by signaling through the glial cell line-derived neurotrophic factor family receptor alpha-like (GFRAL) located in specific regions of the brainstem [5]. This interaction is believed to contribute to weight loss, sarcopenia, and cachexia in cancer patients [39, 40]. Antibody-mediated inhibition of GDF15-GFRAL activity was shown to reverse excessive lipid oxidation and prevent sarcopenia and cachexia in mice with cancer [9]. Sarcopenia is characterized by a progressive and generalized decrease in skeletal muscle mass and is often caused by various chronic conditions such as aging, inactivity, chronic lung disease, and cancer. Several studies have reported that the prevalence of sarcopenia in patients with CRC is relatively high [41-44]. In a study by Broughman et al., in 87 patients with stage I-III CRC who were older than 70 years, 60% of men and 56% of women had preoperative myopenia; the total amount of muscle was measured on CT images and the skeletal muscle index was determined on the


A
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BMI
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GDF-15 expression in CRC tissues
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Preoperative serum GDF-15 levels
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Rho=0.16
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Preoperative serum GDF-15 levels
Preoperative serum CRP levels
Rho=0.42
P<0.0001
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Preoperative serum GDF-15 levels
P=0.008
BMI Low
BMI High
N
Preoperative serum GDF-15 levels
P<0.0001
PMI Low
PMI High
O
Preoperative serum GDF-15 levels
P=0.03
IMAC Low
IMAC High
P
Preoperative serum GDF-15 levels
P<0.0001
Low
High
Preoperative serum CRP levels

Figure 3. A-D. Scatter plots of GDF-15 expression in cancerous tissues and preoperative BMI, PMI, IMAC, and CRP levels. E-H. Scattered boxplots of GDF-15 expression in cancerous tissues categorized by high and low BMI, PMI, IMAC, and CRP. I-L. Scatter plots of serum GDF-15 levels and preoperative BMI, PMI, IMAC, and CRP levels. M-P. Scattered boxplots of serum GDF-15 levels categorized by high and low BMI, PMI, IMAC, or CRP. GDF-15 expression in cancerous tissues was measured using qRT-PCR, and serum GDF-15 levels were determined by ELISA. BMI was calculated as body weight (kg) divided by height squared (m^2). PMI and IMAC were assessed from preoperative computed tomography (CT) images at the level of the fourth lumbar vertebra, with PMI defined as the cross-sectional area of the bilateral psoas muscles (cm^2) divided by height squared (m^2), and IMAC calculated as the ratio of the mean CT value of the multifidus muscle to that of subcutaneous fat. CRP levels were obtained from routine blood tests. BMI, body mass index; CRP, C-reactive protein; GDF-15, growth differentiation factor 15; IMAC, intramuscular adipose tissue content; PMI, psoas muscle mass index.

Table 3. Multivariate analysis for risk factors of high serum GDF-15 levels[†] in CRC patients

Variables	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Gender (Male)	1.66	0.93-2.96	0.09			
Age (>66 years[‡])	3.68	2.01-6.72	<0.0001*	5.08	2.40-10.74	<0.0001*
Histological type (Undifferentiated)	1.76	0.68-4.54	0.24			
Location (Left)	0.81	0.44-1.48	0.49			
T classification (pT3/4)	2.23	1.13-4.39	0.02*	1.51	0.67-3.39	0.32
Venous invasion (Present)	1.05	0.58-1.91	0.86			
Lymphatic invasion (Present)	0.85	0.48-1.51	0.57			
Lymph node metastasis (Present)	1.0	0.57-1.76	1.0			
Distant metastasis (Present)	2.02	1.05-3.88	0.04*	1.55	0.65-3.69	0.32
Preoperative BMI levels (Low[§])	1.83	1.03-3.25	0.04*	1.59	0.79-3.23	0.2
Preoperative PMI levels (Low[†])	2.56	1.34-4.90	0.004*	2.09	0.95-4.61	0.07
Preoperative IMAC levels (Low[†])	1.44	0.79-2.63	0.24			
Preoperative serum CRP levels (High[§])	6.55	3.35-12.80	<0.0001*	7.69	3.44-17.18	<0.0001*

*P<0.05. BMI: body mass index; CI: confidence interval; CRC: colorectal cancer; CRP: C-reactive protein; GDF-15: growth differentiation factor 15; IMAC: intramuscular adipose tissue content; OR: odds ratio; PMI: psoas muscle mass index. [†]Cutoff thresholds for preoperative serum GDF-15 levels, PMI, and IMAC were individually determined by receiver operating characteristic curve analysis using Youden's index for disease-free survival in CRC patients. GDF-15 was determined for all patients, while PMI and IMAC were determined separately for males and females. [‡]The median age at surgery was 66 years in this cohort. [§]Cutoff for BMI was set at 21, for CRP at 0.5 mg/dL (5.0 mg/L).

basis of patient height [41]. Growing evidence indicates that sarcopenia has substantial clinical impacts on patients with CRC, contributing to a heightened risk of treatment-related adverse events, postoperative complications, and poor survival outcomes [16, 45, 46]. Thus, the clinical significance of myopenia and sarcopenia in CRC patients is attracting growing interest in the research community.

Previous studies have revealed a significant correlation between GDF-15 and several clinicopathological factors associated with disease progression, including TNM stage progression in CRC patients [11]. Additionally, some studies from North America, Oceania, and Asia have demonstrated a clear association between GDF-15 and oncological outcomes in CRC patients [11, 47-49]. However, the association between GDF-15 and host factors in CRC patients in the preoperative setting is unclear. Therefore, this study not only focused on the relationship between oncological outcomes and preoperative GDF-15 levels in CRC patients but also explored correlations between GDF-15 and various body composition indicators such as BMI, PMI, and IMAC, as well as the inflammatory marker CRP. Furthermore, as a novel approach, this study performed direct triangular comparison between tissue- or serum-GDF-15 and host factors, including inflammation and body composition status, in CRC patients using matched pair samples and clinical data. These analyses revealed several novel discoveries regarding the clinical relevance of GDF-15 in CRC patients. First, we found that

GDF-15 was significantly overexpressed in CRC tissues compared with adjacent normal mucosa. Second, while no significant correlation was observed between GDF-15 expression levels in CRC tissues and clinicopathological factors in CRC patients, high GDF-15 preoperative serum level was significantly associated with older age and several clinicopathological factors indicative of disease progression, including advanced pathological T category, presence of distant metastasis, and advanced TNM stage classification in CRC patients. Third, while no significant association between GDF-15 expression levels in CRC tissues and oncological outcome in CRC patients was observed, elevated GDF-15 preoperative serum level was an independent prognostic factor for both DFS and OS in CRC patients. Fourth, while there was no significant correlation between GDF-15 expression in CRC tissues and host factors, including body composition status or preoperative CRP in CRC patients, high GDF-15 preoperative serum level was significantly associated with preoperative low BMI, myopenia (low PMI), myosteatosis (high IMAC), and increased CRP levels. Finally, multivariate analysis demonstrated that increased CRP was an independent predictive factor for high serum GDF-15 concentration in these patients. These findings are consistent with recent research on the role of GDF-15 in sarcopenia or cachexia and may serve as additional evidence supporting the inclusion of systemic inflammation as one of the recent diagnostic criteria for malnutrition or cachexia. The SIR accompanied by elevated CRP via the tumor-host interaction may be one of the major drivers of increased serum GDF-15 concentrations and myopenia in CRC patients. Measuring preoperative serum GDF-15 levels could potentially identify patients who need more robust nutritional interventions and rehabilitation targeted at preoperative sarcopenia.

Future studies should further focus on elucidating the detailed mechanisms by which GDF-15 contributes to cancer progression and various host metabolic responses. Additionally, integrating GDF-15 with inflammatory markers such as CRP and body composition indicators may improve prognostic accuracy in CRC patients. Since the relationship between GDF-15 and host factors such as body composition may vary across geographical populations, validating its clinical significance in diverse regions is essential. Developing a comprehensive risk stratification model based on these findings could further refine perioperative management strategies.

Conclusion

In conclusion, our research highlights the clinical utility of preoperative serum GDF-15 as a highly reliable biomarker that strongly reflects host factors and robustly predicts oncological outcomes in CRC patients. Evaluation of preoperative serum GDF-15 levels may help assist physicians to design more effective perioperative management approaches and enhance postoperative oncological follow-up strategies for CRC patients.

Acknowledgements

The authors thank the medical and co-medical staff at Mie University Hospital for their cooperation. The authors thank Gabrielle White Wolf, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure of conflict of interest

None.

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