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Low Serum Interleukin-13 Levels Correlate with Poorer Prognoses for Colorectal Cancer Patients

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Interleukin-13 (IL-13) is an immunosuppressive cytokine produced by several immune cells and cancer cells. The aim of this retrospective study was to determine if serum IL-13 levels have an association with clinical outcome in patients with colorectal cancer. A total of 241 patients with colorectal cancer were enrolled in the present study. Preoperative serum IL-13 concentrations were measured by enzyme-linked immunosorbent assay. We analyzed the association of serum IL-13 levels with clinicopathological variables. Patients with lymph node metastasis, lymphatic invasion, vascular invasion, distant metastases or advanced stage of disease had significantly lower serum IL-13 levels. Low serum IL-13 was significantly associated with both poor recurrence-free and overall survival. Multivariate analysis showed that low IL-13 levels were an independent predictive marker for poor prognosis. In conclusion, our data suggest that low serum IL-13 levels may be a useful predictive marker for poor prognosis in colorectal cancer.

Key word: Serum IL-13 – Colorectal cancer – Recurrence – Prognosis

Introduction

Colorectal cancer is a prevalent cancer worldwide and the second most common cause of cancer-related mortality. In developed countries, the 5-year survival rate is approximately 40%. Metastases to the liver and lung are the main causes of death in colorectal cancer.[1] Identifying predictive markers for cancer progression and prognosis would aid in improving the clinical outcome and potential treatment stratification for patients with colorectal cancer.

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Interleukin-13 (IL-13), a 33-amino acid peptide, is a Th2 family cytokine. IL-13 is secreted by adaptive effecter CD4 T cells, innate immune cells such as eosinophils, basophils, mast cells, dendritic cells, natural killer (NK) cells, and NK T cells, and exerts anti-inflammatory properties. Several authors have reported the association of IL-13 with clinical outcomes in patients with systemic inflammatory response syndrome and sepsis.[2,3] In human diseases, the most prominent and well-studied role of IL-13 is in allergic asthma.[4] In gastroenterology, IL-13 has been reported to be involved in the pathogenesis of ulcerative colitis,[5] Crohn disease, intestinal fistula and fibrosis formation caused by Crohn disease,[6] hepatic fibrosis,[7] and eosinophilic esophagitis.[8,9] Moreover, recent reports have demonstrated IL-13 secretion by cancer cells.[10] The high-affinity IL-13 receptor, IL-13Rα2, was reported to be a novel tumor-associated antigen on colorectal cancer cells that impacted adhesion, migration, invasion, metastatic colonization, and prognosis.[11] Therefore, the control of IL-13 has received a great deal of attention as a new therapeutic strategy for gastrointestinal diseases in recent years. Indeed, preclinical trials for asthma and perianal fistulas induced by Crohn disease and eosinophilic esophagitis using an IL-13 inhibitor are ongoing.[6]

Several studies have demonstrated that colorectal cancer itself has a direct immunosuppressive effect.[12,13] Immunosuppression occurs at both a molecular and cellular level, with tumor immune tolerance starting gradually and locally, then progressing and finally spreading to the whole organism.[14] However, the contributions of IL-13 to antitumor effects in colorectal cancer are poorly understood.

The aim of this retrospective study is to determine if there is an association between preoperative serum IL-13 levels and clinical outcomes in colorectal cancer.

Materials and Methods

Patients and blood sampling

A total of 241 patients (148 men and 93 women; median age 67 years; age range: 12–92 years) who underwent resection of colorectal cancer at the department of gastrointestinal and pediatric surgery of the Mie University Graduate School of Medicine from 1998 to 2007, and for whom preoperative serum could be collected, were enrolled in this retrospective study. Preoperative chemotherapy including hepatic arterial infusion was given to 13 patients and 42 patients with rectal cancer had preoperative chemoradiotherapy. There was no perioperative mortality among the patients. All patients were classified according to Union for International Cancer Control (UICC) stage classifications postoperatively. The pathological T stages were pT1 (n = 19); pT2 (n = 42); pT3 (n = 125); and pT4 (n = 55). Patients (n = 104; 43%) patients had lymph node metastases. Tumors (n = 220) had well-differentiated, moderately differentiated adenocarcinomas, and the remainder had poorly differentiated, mucinous adenocarcinomas and signet ring cell carcinomas. There were 46 patients with stage I, 70 patients with stage II, 63 patients with stage III, and 62 patients with stage IV disease. The median follow-up period was 57 months (range: 1–142 months).

Blood samples were obtained before surgery. Each sample was centrifuged at 3000*g* for 5 minutes and then frozen at –80°C until analysis. All of the patients signed an informed consent for their tissues to be used in this study. The study was performed in accordance with the Helsinki Declaration and was approved by the ethics review board at Mie University Hospital.

Determination of serum IL-13 levels

Serum IL-13 levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human IL-13 ELISA kit; RayBiotech, Norcross, Georgia; detection range: 0.15–40 pg/mL), according to the manufacturer's instructions. Serum samples (50 μL) were incubated at room temperature for 2.5 hours, and 96-well plates were incubated with standards at different concentrations. After several washes, wells were incubated with biotinylated antibodies at room temperature for 1 hour, incubated with HRP-conjugated streptavidin for 45 minutes at room temperature, and followed by a one-component substrate (TMB One-step Substrate Reagent; [RayBiotech, Norcross, Georgia]) at room temperature for 30 minutes. Enzymatic reactions were developed and absorbance was measured at 450 nm (A450) in a microplate photometer (Multiskan FC; Thermo Scientific, Yokohama, Japan) within 30 minutes after stopping color development. Protein levels were calculated according to standard curves.

Statistical analyses

All statistical analyses were performed using a statistics application (StatView 5.0 for Windows;

Table 1 Association of serum IL-13 levels with clinicopathological variables

Variables		n = 241	%	IL-13, pg/mL	*P* value
Sex					
	Male	148	61	0.622	0.4551
	Female	93	39	0.559	
Age (median: 67 years)					
	< 67	121	50	0.595	0.9462
	≥ 67	120	50	0.639	
Tumor size (median: 40 mm)					
	<50	144	60	0.615	0.8824
	≥50	97	40	0.602	
T classification					
	T1	19	8	1.361	0.0921[a]
	T2	42	17	0.673	
	T3	125	52	0.595	
	T4	55	23	0.535	
Lymph node metastasis					
	Absent	137	57	0.716	0.0023[b]
	Present	104	43	0.509	
Lymphatic invasion					
	Absent	25	10	0.965	0.0032[b]
	Present	216	90	0.590	
Vascular invasion					
	Absent	63	26	0.808	0.0211
	Present	178	74	0.587	
Histology					
	Well/moderately	220	91	0.617	0.5369
	Poorly/signet/mucinous	21	9	0.523	
Stage					
	I	46	19	0.804	0.0038[a]
	II	70	29	0.687	
	III	63	26	0.545	
	IV	62	26	0.502	
Distant metastasis					
	Absent	181	75	0.675	0.0037[b]
	Present	60	25	0.491	

Detail of distant metastasis: liver metastasis, n = 38; lung metastasis, n = 19; peritoneal metastasis, n = 16. Serum IL-13 concentrations are expressed as median value (pg/mL).

[a]Kruskal-Wallis test.

[b]Mann-Whitney *U* test.

SAS Institute, Inc., Cary, North Carolina). Associations between continuous variables and categorical variables were evaluated using the Mann-Whitney *U* test for 2 groups and the Kruskal-Wallis test for 3 or more groups. Recurrence-free survival (RFS) and overall survival (OS) times were calculated from the date of surgery to the date of disease recurrence and death, respectively. A nonparametric receiver operating characteristic (ROC) analysis was performed to calculate the best cutoff value for serum IL-13 concentration that would be predictive of prognosis, using a statistical software package (MedCalc 7.2 for Windows; MedCalc Software, Ostend, Belgium). RFS and OS probabilities were calculated using the Kaplan-Meier product limit method; intergroup differences were determined using a log-rank test. A nonparametric ROC analysis determined that the optimal cutoff value for serum IL-13 concentrations was 0.5127 (sensitivity: 51.7%; specificity: 71.3%) for RFS and 0.1212 (sensitivity: 30.6%; specificity: 85.5%) for OS. RFS and OS probabilities were calculated using the Kaplan-Meier product limit method; intergroup differences were determined using a log-rank test. The influence of disease recurrence and survival predictors identified via univariate analysis was accessed by multivariate analysis using Cox's proportional hazards model. Two-sided *P* values less than 0.05 were considered statistically significant.

Table 2 Uni- and multivariate analyses for predictors of disease recurrence in stage I through III patients

Univariate analysis	HR	95% CI	*P* value
Tumor size (≥50 mm vs. < 50 mm)	1.074	0.498–2.317	0.8562
T classification (T3,T4 vs. T1,T2)	7.710	1.828–32.525	0.0054
Lymph node metastasis (positive vs. negative)	1.640	0.787–3.415	0.1865
Lymphatic invasion (positive vs. negative)	1.061	0.368–3.059	0.9127
Vascular invasion (positive vs. negative)	1.805	0.734–4.434	0.1981
Histology (well/moderately vs. poorly/signet/mucinous)	0.907	0.215–3.821	0.8937
IL-13 (<cutoff vs. ≥cutoff)	2.241	1.079–4.651	0.0304
Multivariate analysis			
pT classification (T3,T4 vs. T1,T2)	7.197	1.702–30.427	0.0073
IL-13 (<cutoff vs. ≥cutoff)	1.993	0.959–4.142	0.0645

CI, confidence interval; HR, hazard ratio.

Results

Association of serum IL-13 with clinicopathological variables

Table 1 shows the association between serum IL-13 levels and clinicopathological variables. Serum IL-13 levels were significantly lower in patients with lymph node metastasis ($P = 0.0023$), lymphatic invasion ($P = 0.0032$), vascular invasion ($P = 0.0211$), or distant metastasis ($P = 0.0037$) than patients without them. Serum IL-13 levels were gradually decreased in accordance to UICC staging ($P = 0.0038$).

Association between serum IL-13 and survival

Colorectal cancer patients were divided into 2 groups according to the best predictive cutoff value determined by ROC analysis in order to examine the predictive value of serum IL-13. In 179 patients with stage I–III disease with curative intent, low serum IL-13 levels were significantly associated with poor RFS ($P = 0.0262$, log-rank test; Fig. IA). On the basis of Cox's univariate proportional hazards analysis, pathological T stage (T3/4; $P = 0.0054$) and low serum IL-13 ($P = 0.0304$) were both significant prognostic factors for disease recurrence. However, multivariate analysis did not identify serum IL-13 levels as an independent predictive factor for disease recurrence (Table 2). Furthermore, patients with low serum IL-13 levels had significantly poorer prognoses than those with high IL-13 levels when examined across all patients ($P = 0.0008$, log-rank test; Fig. 1B). On the basis of Cox's univariate proportional hazards analysis, tumor size (≧50 mm; $P = 0.0021$), pathological T stage (T3/4; $P = 0.0003$), lymph node metastasis ($P = 0.0002$), vascular invasion ($P = 0.0065$), distant metastasis ($P < 0.0001$), and low serum IL-13 ($P =$

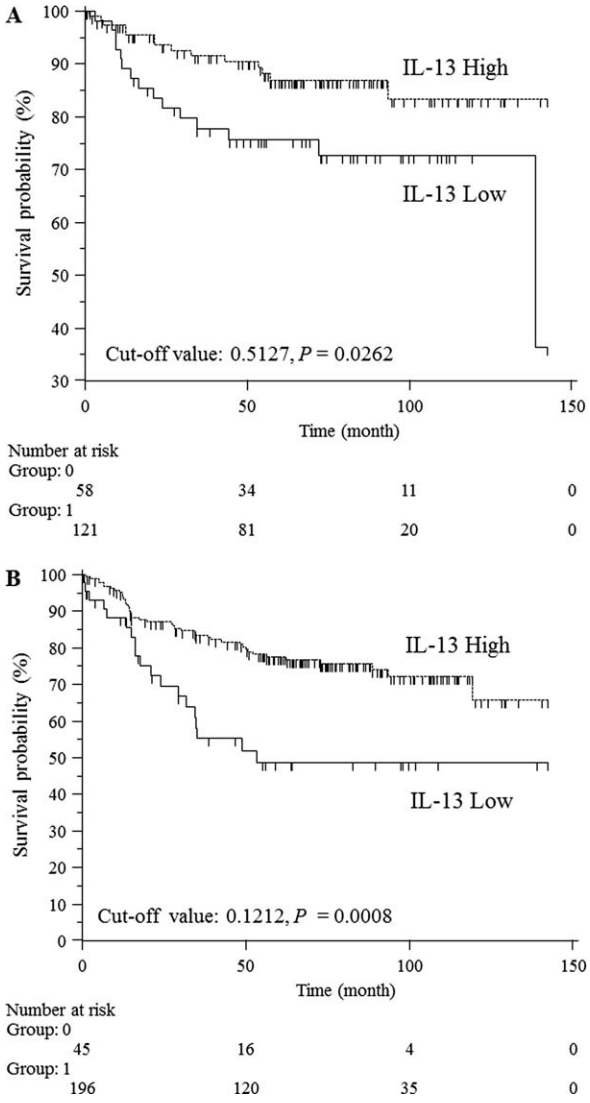

Fig.1 (A) Recurrence-free survival according to serum IL-13 levels in stage I-III patients with curative contents (n = 179). (B) Overall survival according to serum IL-13 levels in all patients (n = 241). The *P* value was determined using the log-rank test.

Table 3 Uni- and multivariate analyses for predictors of survival in all patients

Univariate analysis	HR	95% CI	*P* value
Tumor size (≥50 mm vs. <50 mm)	2.204	1.333–3.644	0.0021
T classification (T3,T4 vs. T1,T2)	6.638	2.408–18.300	0.0003
Lymph node metastasis (positive vs. negative)	2.682	1.606–4.476	0.0002
Lymphatic invasion (positive vs. negative)	2.753	0.862–8.784	0.0872
Vascular invasion (positive vs. negative)	2.980	1.356–6.546	0.0065
Histology (well/moderately vs. poorly/signet/mucinous)	1.274	0.548–2.959	0.5736
Distant metastasis (positive vs. negative)	9.175	5.466–15.403	<0.0001
IL-13 (<cutoff vs. ≥cut-off)	2.558	1.499–4.366	0.0006
Multivariate analysis			
T classification (T3,T4 vs. T1,T2)	3.577	1.242–10.301	0.0182
Lymph node metastasis (positive vs. negative)	1.864	1.104–3.148	0.0199
Distant metastasis (positive vs. negative)	6.241	3.617–10.769	<0.0001
IL-13 (<cutoff vs. ≥cutoff)	1.816	1.046–3.153	0.0341

0.0006) were all significant prognostic factors for survival. Multivariate analysis identified advanced T stage ($P = 0.0182$), distant metastasis ($P < 0.0001$), and low serum IL-13 levels ($P = 0.0341$) as independent risk factors for predicting poor prognosis (Table 2).

Discussion

In this study, we found that preoperative serum IL-13 was inversely correlated with factors reflecting tumor progression, and that decreased serum IL-13 was an independent predictive factor for poor prognosis in patients with colorectal cancer. To the best of our knowledge, this is the first report to describe an association of serum IL-13 levels with clinical outcomes in colorectal cancer patients. Formentini *et al* reported that low immunoreactivity of IL-13 in colorectal cancer was an independent prognostic factor for overall survival.[15] Moreover, several studies have shown that IL-13 inhibits cancer cell proliferation.[16–18] Conversely, IL-13 has been implicated to promote cancer cell proliferation, and IL-13 has been associated with lymph node metastasis in several malignancies.[10,19–21] Recently, Barderas *et al* reported that IL-13Rα2 was involved in tumor progression and metastasis because silencing of IL-13Rα2 led to the promotion of apoptosis via suppression of AKT activation and decreased cell adhesion, invasion, and clonogenicity in colon cancer cells.[11] Moreover, Formentini *et al* reported that neutralization of IL-13 inhibited the growth of pancreatic cancer cells and described that IL-13 could act as an autocrine growth factor in pancreatic ductal adenocarcinoma.[10] IL-13 binds to two cell surface receptors: IL-13Rα1, which dimerizes with IL-4Rα, and IL-13Rα2, which is considered to be a decoy receptor. IL-13Rα2 can serve as a negative regulator of IL-13, since IL-13 exposure leads to transcriptional upregulation and expression, and IL-13Rα2 rapidly cycles from intracellular compartments to capture and internalize IL-13, and IL-13Rα2 expression is inversely related to IL-13/STAT6 activation. Moreover, IL-13 signaling via the JAK/STAT pathway can induce suppressor of cytokine signaling proteins that attenuate the IL-13 signal itself.[6] A review of the IL-13 signaling literature leads us to conclude that IL-13 signaling is complex, and it is not well established that the IL-13 signaling pathway is associated with cancer growth and progression.

Ma *et al* showed that IL-13 reduced the tumorigenicity of B16F1 melanoma and MethA fibrosarcoma cells, most likely through the recruitment of neutrophils and macrophages.[22] Lebel-Binay *et al* found that the anti-tumor effect of IL-13 resulted from pleiotropic effects, including infiltration of non-specific cells such as monocytic cells, macrophages, and neutrophils into the tumor. Furthermore, the authors were able to demonstrate the stimulation of tumor-specific immunity using IL-13-transfected P815 mastocytoma cells.[23] These data highlight a chemotactic function for IL-13 in tumor rejection. The increase of tumor-infiltrating lymphocytes is a well-known predictor of better prognosis for various malignancies, including colorectal cancer.[24,25] Similarly, circulating and infiltrating neutrophils have been identified as strong, independent risk factors for poor outcome in various malignancies.[26]

Thus, the roles of IL-13 are complicated and appear to vary according to the type of malignancy. In the present study, preoperative serum IL-13

levels may reflect systemic response to colorectal cancer rather than local function of IL-13. Therefore, the inhibition of IL-13 for cancer treatment may be a double-edged sword because decreasing IL-13 may lead to the suppression of cancer immunity.

In conclusion, preoperative serum IL-13 was inversely correlated with factors reflecting tumor progression. Our results suggest that low serum IL-13 levels may be a useful predictive marker for poor prognosis in colorectal cancer.

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