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Plasma Cytokines as Potential Response Indicators to Dietary Freeze-Dried Black Raspberries in Colorectal Cancer Patients

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Abstract

Oral consumption of freeze-dried black raspberries attenuated neoplastic changes in colorectal tissue markers of apoptosis, cell proliferation, and angiogenesis in colorectal cancer (CRC) patients. To determine, whether plasma concentrations of interleukin (IL)-1β, IL-2, IL-6, IL-8, IL-10, IL12p70, granulocyte macrophage colony stimulating factor (GM-CSF), interferon-γ, and tumor necrosis factor-α (TNF-α) were associated with berry treatment and changes in colorectal tissue markers of apoptosis, cell proliferation, and angiogenesis, plasma and biopsy samples of adenocarcinoma and adjacent normal-appearing colorectal tissue were collected before and during berry treatment from 24 CRC patients who had not received prior therapy and drank a slurry of black raspberry powder (20 g in 100 ml drinking water) 3 times-a-day for 1-to-9 weeks. Plasma concentrations of GM-CSF (+0.12 ± 0.04 pg/mL; $P = 0.01$) and IL-8 (−1.61 ± 0.71 pg/mL; $P = 0.04$) changed in patients receiving berries for more than 10 days. These changes were correlated with beneficial changes in markers of proliferation ($r_{\Delta GM\text{-}CSF,\ \Delta Ki67\ carcinoma\ -\ normal} = -0.51$) and apoptosis ($r_{\Delta IL\text{-}8,\ \Delta TUNEL\ carcinoma\ -\ normal} = -0.52$) observed in colorectal tissue taken within the same week. Plasma concentrations of GM-CSF and IL-8 may serve as non-invasive indicators to monitor tissue response to berry-based interventions for CRC.

Keywords

black raspberries; cytokines; biomarkers; colorectal cancer; prevention

INTRODUCTION

Colorectal cancer (CRC) is one of the most important public health problems, which in the U.S. results in approximately $6.5 billion treatment costs and nearly 50,000 deaths

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[4]Abbreviations: CRC, colorectal cancer; GM-CSF, granulocyte macrophage colony stimulating factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

annually(1–2). Dietary interventions may provide a safe and cost-effective strategy for CRC prevention or as adjuvant to improve the response to traditional CRC treatment. Berries are widely consumed fruits in Western countries and have been used in traditional medicine, as they are enriched in antioxidants and vitamins(3). Oral consumption of freeze-dried black raspberries showed promise for cancer prevention in animal models and early phase clinical trials(4–10). In two clinical pilot studies, freeze-dried black raspberries decreased colon polyp size in familial adenomatous polyposis patients by 38% and attenuated the progression of CRC tissue markers in CRC patients (i.e., decreasing cell proliferation, angiogenesis and increasing apoptosis)(4–5, 10).

Growing evidence suggests that inflammation and cytokines promotes carcinogenesis, including CRC(11). Thus, circulating cytokine concentrations have been proposed as indicators of risk and stages of CRC(12). In general, CRC is associated with decreased concentrations of interleukin (IL)-2 and increased concentrations of IL-6, IL-8, and tumor necrosis factor (TNF)α; whereas, the results for IL-1β, IL-10, IL-12, granulocyte macrophage colony stimulating factor (GM-CSF), and interferon (IFN)γ have been less conclusive, as previously reviewed(13).

There is a critical need for biomarkers of response to interventions that may be useful as preventive or therapeutic agents for CRC. Changing circulating cytokine concentrations is one of the many molecular mechanisms by which black raspberries may inhibit CRC(8–9). Therefore, the objective of this study was to determine whether changes in plasma concentrations of IL-1β, IL-2, IL6, IL-8, IL-10, IL-12p70, GM-CSF, IFN-γ, and TNF-α were associated with berry treatments and changes in colorectal tissue markers of apoptosis, cell proliferation, and angiogenesis in CRC patients. We hypothesized that oral consumption of black raspberries would result in changes in plasma cytokine concentrations that reflect improvements in colorectal tissue markers and thus, may serve as non-invasive indicators of beneficial colorectal tissue changes in CRC patients consuming berries.

MATERIALS AND METHODS

Human clinical trial

This study was part of a phase I pilot clinical trial in which the effect of oral freeze-dried black raspberries on genetic and epigenetic colorectal tissue biomarkers of CRC, including cell proliferation, apoptosis, angiogenesis, methylation of tumor suppressor genes, and expression of Wnt pathway genes, was evaluated in individuals with CRC. The trial has been described in detail previously (10). Briefly, to be eligible, individuals with CRC had to be over 18 years, required surgical resection of the adenocarcinoma, had a life expectancy of at least 12 weeks, had no serious life-threatening conditions, were not pregnant or lactating, and were instructed not to take non-steroidal anti-inflammatory drugs or receive chemotherapy or radiation therapy during the treatment period. In addition, participants were encouraged to record their daily dietary intake, follow a low-phenolic diet during the course of the study, and to refrain from taking vitamin or herbal supplements to reduce the likelihood that other dietary factors may have contributed to the results. The institutional review boards of the Ohio State University Comprehensive Cancer Center and the University of Texas, San Antonio approved the study, and all participants provided written, informed consent before participations in the trial. This study focuses on the six female and 18 male participants, primarily of Caucasian origin (87.5%), with pathologically confirmed CRC (mean age: 59 yrs; range: 37 to 82 yrs; Table 1). Participants with CRC consumed 20 g of freeze-dried black raspberry powder in a slurry of water 3 times per day for approximately three weeks (range: 7 to 63 days) until surgical resection of the adenocarcinoma (Table 1). Regular phone calls to participants were used to monitor compliance and to check for adverse effects, which ranged from no effect to diarrhea,

improved bowel function, or constipation. To evaluate global versus adenocarcinoma tissue specific effects of raspberry treatment, three samples of adenocarcinoma tissue and three samples of adjacent normal-appearing colorectal tissue were biopsied during colonoscopy at the start of the berry treatment and during surgical resection of the adenocarcinoma at the end of the berry treatment period, respectively. One-half of each biopsy sample was fixed in neutral-buffered formalin and embedded in paraffin and stained for Ki67 (proliferation), TUNEL (apoptosis), and CD105 (angiogenesis) and the other half was frozen in liquid nitrogen for evaluating DNA methylation, the results of which have been published previously (10). Plasma samples were taken before berry treatment and approximately 2 weeks (range: 5 to 63 days; Table 1) within the course of the treatment and stored at −80 °C. The principal reason for taking plasma samples was to ensure patient compliance with the berry treatment by assessing the presence of berry anthocyanins in the blood.

Colorectal tissue and plasma analysis

Plasma cytokine concentrations (IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, GM-CSF, IFN-γ, and TNF-α) were measured by the Clinical Support Laboratory of SAIC Frederick, Inc. (Frederick, MD) using a commercially available multiplex 96-well enzyme-linked immunoabsorbent assay kit (MS6000 Human Pro-Inflammatory 9-Plex Ultra-Sensitive Kit K11007; Meso Scale Diagnostics, Gaithersburg, MD) on a Sector™ Imager 6000 according to the manufacturer's recommendation (Meso Scale Diagnostics, Gaithersburg, MD). All samples and standards were run in duplicate and averaged. Standard calibration curves were run on each plate using standards supplied by Meso Scale Diagnostics with a linear range of 0.2 to 10,000 pg/mL.

Colorectal tissue biomarkers of cell proliferation (Ki67), apoptosis (TUNEL), and angiogenesis (CD105) were quantified by immunohistochemical staining and subsequent computer-assisted image analysis as previously described(8, 10). In short, paraffin-embedded tissues were cut into 4 μm sections and placed on slides. Slides were heated for 1 hr at 60 °C, cooled, deparaffinized, and then rehydrated. To block endogenous peroxidase, slides were incubated for 5 min with a 3% aqueous H_2O_2 solution. Slides were then placed in a vegetable steamer in Dako Target Retrieval Solution for 25 min to retrieve antigens. After letting slides cool for 15 min, the sections were stained for 1 hr at room temperature on a Dako Autostainer with the primary antibodies for Ki67 (dilution 1:150; Dako, Carpinteria, CA), CD105 (dilution 1:100; Lab Vision Products, Kalamazoo, MI), and TUNEL (ApopTag Plus Peroxidase in Situ Apoptosis Detection Kit; Chemicon, Billerica, MA), followed by staining with their respective secondary antibody. Stained tissues were photographed at 200× magnification with a bright-field microscope that was mounted with a high resolution spot camera. Images were analyzed using image analysis software (Simple PCI Imaging Systems, Compix Inc., Irvine, CA). Values for staining intensity at each time point per patient are based on 30 whole crypts of normal epithelium (only lower third of crypt for Ki67) and 30 image fields of tumor tissue.

Statistical analysis

Statistical analyses were performed using Statistical Analysis Systems, version 9.1 (SAS, Inc., Cary, NC) software. Changes in plasma cytokines and colorectal tissue markers pre- and during berry treatment were measured using paired *t*-tests. Associations between plasma cytokine values and colorectal tissue markers of proliferation, apoptosis, and angiogenesis were measured using Spearman's correlation coefficients. To evaluate potential effect modification, data were stratified by length of intervention period before blood samples were taken (≤8 days versus >10 days; none of the patients had 9 or 10 days), difference between serum and tissue sampling date (within 1 week versus at least 12 days apart; none of the patients had a difference between 8 and 11 days), baseline values, gender, and age.

All *p*-values correspond to two-sided tests and were considered to be significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

There is a critical need for identification of non-invasive biomarkers that can predict individuals more likely to benefit from a chemopreventive or therapeutic agent for CRC. This is the first study to examine whether plasma cytokines are associated with berry treatment and changes in colorectal tissue markers of apoptosis, cell proliferation, and angiogenesis.

Overall, the berry intervention increased plasma GM-CSF concentrations (+0.12 ± 0.04 pg/mL; $P = 0.007$), whereas the other 8 measured plasma cytokines were not significantly altered (Table 2). The length between start of the berry intervention and blood sampling was inversely associated with plasma concentrations of IL-8 ($r_{\Delta IL\text{-}8,\ length\ of\ berry\ intervention\ in\ days} = -0.44$; $P = 0.03$) and IL-1β ($r_{\Delta IL\text{-}1\beta,\ length\ of\ berry\ intervention\ in\ days} = -0.44$; $P = 0.03$). Because we did not expect to observe changes in plasma cytokine values in the 9 CRC patients whose blood samples had been taken within the first 8 days of the intervention, we focused our analysis on the other 15 CRC patients that had been on the berry intervention for more than 10 days when their blood samples had been taken. We observed that the berry treatment increased plasma concentrations of GM-CSF (+0.12 ± 0.04 pg/mL; $P = 0.01$) in patients receiving berries for more than 10 days (Table 2). Little is known about the effect of raspberries on GM-CSF except that quercetin, a raspberry component, stimulates GM-CSF secretion and recruitment of dendritic cells to human prostate cancer cells(14). Patients receiving berries for more than 10 days also had decreased plasma concentrations of IL-8 (−1.61 ± 0.71 pg/mL; $P = 0.04$; Table 2). Consistent with our results, raspberries attenuated IL-8 secretion in cell culture(15). Furthermore, supplementation of quercetin, a component of raspberries, in humans attenuated liposaccharide-induced IL-8 secretion(16). Thus, changes in circulating GM-CSF and IL-8 may indicate a treatment response in CRC patients consuming berries.

Berry treatment modified protectively colorectal tissue markers of proliferation (Ki67), apoptosis (TUNEL), and angiogenesis (CD105) in adenocarcinomas and adjacent normal appearing tissue (Table 2). Berry treatment decreased Ki67 staining in adenocarcinoma (−6.60 ± 1.82%; $P = 0.001$) and adjacent normal appearing tissue (−3.56 ± 1.26%; $P = 0.01$) and increased TUNEL staining in adenocarcinoma tissue (+2.70 ± 1.22 pg/mL; $P = 0.04$). In addition, berry treatment tended to decrease CD105 staining in adjacent normal appearing tissue (−0.91 ± 0.48%; $P = 0.07$). Berries may exert part of their chemoprotective effects by inhibiting cell proliferation and angiogenesis, while promoting apoptosis(3, 5). The effects of raspberries on proliferation, apoptosis, and angiogenesis may not be limited to adenocarcinoma tissue but may extend to adjacent normal-appearing colorectal tissue (Table 2). Adjacent colorectal tissue, albeit visually of normal appearance, may support the growth of the adenocarcinoma as tumor microenvironment. Therefore, the anti-proliferative and anti-angiogenic effect of the berry-based intervention in the adjacent normal-appearing colorectal tissue may contribute to shrinking of the tumor(4).

Overall, changes in plasma cytokine concentrations during the berry intervention were not associated with changes in colorectal tissue markers of proliferation, apoptosis, and angiogenesis (Table 3), with the exception of TNF-α and apoptosis in normal-appearing tissue ($r_{\Delta TNF\text{-}\alpha,\ \Delta TUNEL\ normal} = -0.60$; $P = 0.002$). Because the goal of the study was to evaluate whether plasma cytokine concentrations reflected the current status of colorectal tissue markers, we excluded the results of the 7 CRC patients whose plasma and colorectal tissue samples had been taken at least 12 days apart. We focused our analysis on the results

of the other 17 CRC patients whose plasma and colorectal tissue samples had been taken within one week (Table 3). Decreased proliferation of adenocarcinoma compared to adjacent normal appearing tissue was associated with increased plasma concentrations of IL-12p70 ($r_{\Delta IL\text{-}12p70,\ \Delta Ki67\ carcinoma\ -\ normal} = -0.48$; $P = 0.05$) and GM-CSF ($r_{\Delta GM\text{-}CSF,\ \Delta Ki67\ carcinoma\ -\ normal} = -0.51$; $P = 0.03$), respectively. Granulocyte macrophage colony stimulating factor can activate the immune response against tumors(17) by promoting proliferation and differentiation of hematopoietic stem cells into dendritic cells(18–19).

Decreased plasma IL-8 concentrations were associated with increased apoptosis of adenocarcinoma ($r_{\Delta IL\text{-}8,\ \Delta TUNEL\ carcinoma} = -0.50$; $P = 0.04$) and increased apoptosis of adenocarcinoma versus adjacent normal appearing tissue ($r_{\Delta IL\text{-}8,\ \Delta TUNEL\ carcinoma\ -\ normal} = -0.52$; $P = 0.03$; Table 3). IL-8 is a pro-inflammatory chemokine, primarily involved in trafficking of white blood cells(20), that may promote carcinogenesis by decreasing apoptosis of endothelial and cancer cells(21). Elevated expression of IL-8 in the human tumor microenvironment is one of the strongest predictors of tumor progression, metastasis, invasion, and recurrence(22). Regardless whether IL-8 result in the above mentioned changes or vice versa(20), our results suggest that changes in plasma concentrations of GM-CSF and IL-8 are associated with changes in colorectal tissue markers in response to berry treatment.

Strengths of the current study are the high compliance rates combined with little or no side effects, the standardized composition of the raspberries, and the ability to evaluate colorectal tissue biomarkers before and during the berry intervention(4–5, 10). One limitation is the variable metabolism and absorption rates of bioactive berry components(3). Other limitations are the small number of participants, the age variability of participants, lack of tissue markers of inflammation in the trial, such as COX-2 and NF-κB, plasma cytokines not being a primary outcome of the trial, multiple comparisons, short treatment period, and the time difference between blood and biopsy sampling for some participants. We tried to account for these limitations by focusing on participants who consumed berries over 10 days and had a blood sample within one week of the biopsy sample, respectively. The time dependent change of plasma IL-8 suggests that a longer intervention may result in larger concentration changes and potentially more cytokines being altered, as had been observed in animal models(9).

Combining several cytokine markers in plasma may provide a more detailed picture of treatment effects; however, it did not provide additional information in this study (data not shown). One reason is that plasma cytokine concentrations have inherent limitations as they are not specific to location, can be variable in time length and fold-change, fluctuate over day, and are influenced by multiple other factors.

In conclusion, our results suggest that changes in plasma GM-CSF and IL-8 concentrations may serve as indicators of beneficial response to berry treatment and thus, may be useful as a means to monitor response to berry-based interventions for CRC. Verification of these results in larger studies with longer treatment periods and parallel blood and tissue sampling of inflammation markers are needed to clarify the role and clinical utility of changes of plasma cytokine concentrations as a subset of indicators for monitoring response to oral raspberry treatment for CRC.

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TABLE 1

Participant characteristics

Participant Number	Gender	Age (years)	Carcinoma Location	Lymphnode Involved	Endpoint	Berry Treatment Plasma (days)	Berry Treatment Biopsy (days)	Compliance (%)
1	Male	77	Rectal	No	Chemoradiation	5	17	98
2	Female	46	Rectal	No	Surgery	8	27	100
3	Male	55	Rectal	No	Surgery	7	19	98
4	Male	71	Ascending Colon	No	Surgery	12	16	100
5	Male	50	Rectal	No	Chemoradiation	15	15	100
6	Male	69	Ascending Colon	No	Surgery	13	27	98
7	Male	53	Rectal	No	Chemoradiation	26	27	96
8	Female	58	Rectal	Yes	Chemoradiation	19	20	73
9	Female	52	Rectal	No	Chemoradiation	5	20	62
10	Male	51	Rectal	No	Surgery	34	34	94
11	Male	55	Rectal	No	Chemoradiation	25	27	99
12	Male	41	Rectal	Yes	Chemoradiation	5	19	100
13	Male	57	Rectal	Yes	Chemoradiation	7	14	100
14	Male	65	Rectal	No	Chemoradiation	8	15	98
15	Male	72	Rectal	No	Chemoradiation	14	27	99
16	Male	82	Rectal	No	Chemoradiation	12	21	83
17	Male	55	Rectal	No	Chemoradiation	26	26	100
18	Female	66	Rectal	No	Chemoradiation	15	15	100
19	Male	37	Rectal	No	Chemoradiation	7	7	100
20	Male	72	Ascending Colon	No	Surgery	29	29	100
21	Male	49	Rectal	No	Chemoradiation	19	31	100
22	Female	47	Descending Colon	No	Unknown	63	63	100
23	Female	51	Rectal	No	Chemoradiation	11	11	100
24	Male	72	Rectal	No	Chemoradiation	15	15	96

TABLE 2

Effect of berry-based intervention on plasma cytokines and colorectal tissue markers in colorectal cancer patients

Variable	Berry (Pre)	Berry (Post)	ΔBerry (Post - Pre)	*P*-value
Overall (n =24):	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Plasma cytokines (in pg/mL):				
Interleukin-1β	1.55 ± 1.02	1.81 ± 1.18	0.27 ± 0.17	0.13
Interleukin-2	1.37 ± 0.43	1.41 ± 0.39	0.04 ± 0.11	0.70
Interleukin-6	7.83 ± 4.02	7.38 ± 3.74	−0.45 ± 0.51	0.38
Interleukin-8	9.16 ± 1.17	8.72 ± 1.16	−0.45 ± 0.60	0.46
Interleukin-10	3.98 ± 0.65	3.86 ± 0.56	−0.11 ± 0.15	0.46
Interleukin-12p70	3.38 ± 0.73	3.28 ± 0.62	−0.11 ± 0.15	0.49
GM-CSF[a]	0.89 ± 0.23	1.01 ± 0.23	0.12 ± 0.04	0.007
Interferon-γ	11.5 ± 9.63	11.3 ± 9.65	−0.20 ± 0.39	0.60
TNF-α[a]	16.3 ± 7.90	16.2 ± 8.10	−0.06 ± 0.32	0.85
Colorectal tissue markers:				
Proliferation (Ki67; % staining)				
Carcinoma	57.8 ± 2.35	51.2 ± 1.87	−6.60 ± 1.82	0.001
Normal[b]	11.7 ± 1.40	8.17 ± 1.11	−3.56 ± 1.26	0.01
Carcinoma - Normal[2]	46.0 ± 2.74	43.0 ± 2.35	−3.05 ± 1.74	0.09
Apoptosis (TUNEL; % staining)				
Carcinoma	4.37 ± 0.99	7.07 ± 1.22	2.70 ± 1.22	0.04
Normal[b]	0.59 ± 0.10	0.80 ± 0.17	0.21 ± 0.15	0.18
Carcinoma - Normal	3.78 ± 0.97	6.27 ± 1.22	2.49 ± 1.21	0.05
Angiogenesis (CD 105; % staining)				
Carcinoma	6.74 ± 1.66	4.14 ± 0.80	−2.60 ± 1.79	0.16
Normal[b]	2.50 ± 0.39	1.59 ± 0.28	−0.91 ± 0.48	0.07
Carcinoma - Normal	4.24 ± 1.59	2.55 ± 0.62	−1.68 ± 1.67	0.32
Over 10 treatment days (n = 15):				
Interleukin-1β	0.57 ± 0.11	0.62 ± 0.11	0.05 ± 0.08	0.55
Interleukin-2	0.88 ± 0.18	0.95 ± 0.25	0.06 ± 0.12	0.61
Interleukin-6	4.04 ± 0.85	3.62 ± 1.02	−0.42 ± 0.59	0.49
Interleukin-8	8.58 ± 1.42	6.98 ± 1.06	−1.61 ± 0.71	0.04
Interleukin-10	3.62 ± 0.70	3.48 ± 0.62	−0.13 ± 0.16	0.41
Interleukin-12p70	2.76 ± 0.59	2.72 ± 0.55	−0.04 ± 0.13	0.76
GM-CSF[a]	0.56 ± 0.13	0.68 ± 0.13	0.12 ± 0.04	0.01
Interferon-γ	2.01 ± 0.58	1.53 ± 0.43	−0.48 ± 0.61	0.45
TNF-α[a]	8.87 ± 1.04	8.36 ± 1.08	−0.51 ± 0.39	0.21

[a]GM-CSF: granulocyte macrophage colony stimulating factor; TNF-α: tumor necrosis factor α.

[b]Normal refers to normal-appearing colorectal tissue biopsied adjacent to the adenocarcinoma tissue.

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TABLE 3

Associations between changes (Δ = post – pre) in plasma cytokine concentrations and colorectal tissue markers

	Proliferation (ΔKi67)			Apoptosis (ΔTUNEL)			Angiogenesis (ΔCD103)		
ΔCytokines (pg/mL)	**Carcinoma**	**Normaf[a]**	**Carcinoma - Normaf[a]**	**Carcinoma**	**Normaf[a]**	**Carcinoma - Normaf[a]**	**Carcinoma**	**Normaf[a]**	**Carcinoma - Normaf[a]**
Overall (n = 24):									
Interleukin-1β	0.18	0.21	0.05	−0.16	−0.33	−0.16	0.19	0.09	0.14
Interleukin-2	0.01	0.18	−0.08	−0.08	0.05	−0.06	−0.02	−0.28	0.08
Interleukin-6	0.00	0.17	−0.06	0.11	0.02	0.11	−0.37	−0.25	−0.24
Interleukin-8	−0.11	0.10	−0.21	−0.21	0.06	−0.23	0.00	0.16	−0.05
Interleukin-10	0.06	0.26	−0.03	0.14	−0.04	0.13	−0.01	−0.24	0.08
Interleukin-12p70	−0.14	0.21	−0.33	−0.10	−0.06	−0.11	0.22	−0.01	0.23
GM-CSF[b]	−0.22	0.23	−0.35	−0.37	0.06	−0.35	0.14	−0.09	0.25
Interferon-γ	−0.13	0.34	−0.28	−0.14	−0.23	−0.16	0.37	0.19	0.38
TNF-a[b]	0.04	0.26	−0.09	−0.11	−0.60**	−0.13	0.19	0.11	0.16
Blood and tissue sample within 1 week (n = 17):									
Interleukin-1β	0.20	0.26	−0.01	−0.32	−0.35	−0.32	0.16	0.01	0.15
Interleukin-2	−0.06	0.42	−0.34	0.08	0.27	0.10	0.04	−0.12	0.07
Interleukin-6	0.10	−0.09	0.25	0.16	0.21	0.17	−0.46	−0.40	−0.31
Interleukin-8	−0.08	0.05	−0.21	−0.50*	−0.08	−0.52*	−0.08	−0.00	−0.02
Interleukin-10	0.14	0.23	0.09	0.30	0.04	0.33	−0.10	−0.22	−0.06
Interleukin-12p70	−0.15	0.26	−0.48*	−0.00	−0.09	0.00	0.15	0.02	0.07
GM-CSF[b]	−0.31	0.25	−0.51*	−0.38	0.04	−0.37	0.10	−0.17	0.21
Interferon-γ	−0.01	0.15	−0.07	−0.17	−0.27	−0.19	0.39	0.07	0.42
TNF-α[b]	0.16	0.14	0.08	−0.13	−0.71**	−0.14	0.25	0.11	0.22

[a] Normal refers to normal-appearing colorectal tissue biopsied adjacent to the adenocarcinoma tissue.

[b] GM-CSF: granulocyte macrophage colony stimulating factor; TNF-α: tumor necrosis factor α.

* $p<0.05$;

** $p<0.01$