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ARTICLE

A Prospective Study of Macrophage Inhibitory Cytokine-1 (MIC-1/GDF15) and Risk of Colorectal Cancer

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Background Chronic inflammation plays a role in the development of colorectal cancer (CRC). The novel plasma inflammatory biomarker macrophage inhibitory cytokine-1 (MIC-1, GDF15) may have a direct mechanistic role in colorectal carcinogenesis.

Methods We conducted a prospective, nested, case–control study of incident CRC among men and women who provided a prediagnostic blood specimen. We used an enzyme-linked immunosorbent assay to measure MIC-1 and examined associations between quintiles of MIC-1 and CRC using logistic regression adjusted for matching factors (age and date of blood draw), risk factors, and other plasma inflammatory markers. We also assessed the relationship between MIC-1 levels and prostaglandin-endoperoxide synthase 2 (PTGS2)/cyclooxygenase-2 (COX-2) enzyme status in tumors with available tissue for analysis. All statistical tests were two-sided.

Results Compared with men and women within the lowest quintile of plasma MIC-1, the multivariable relative risk (RR) for CRC was 1.93 (95% confidence interval [CI] = 1.27 to 2.94) for the highest quintile ($P_{\text{linear trend}}$ = .004). In an exploratory analysis, we found that among individuals with high plasma MIC-1 levels (quintiles 2–5), compared with nonuse, regular use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) was associated with a lower risk of PTGS2-positive CRC (multivariable RR = 0.60; 95% confidence interval = 0.41 to 0.88) but not PTGS2-negative CRC (multivariable RR = 1.21; 95% CI = 0.71 to 2.07). In contrast, among individuals with low MIC-1 levels (quintile 1), aspirin and NSAID use was not associated with a lower risk of PTGS2-positive CRC (multivariable RR = 0.57; 95% CI = 0.21 to 1.54) or PTGS2-negative CRC (multivariable RR = 1.41; 95% CI = 0.47 to 4.23).

Conclusions Our results support an association between higher levels of circulating MIC-1 (GDF15) and CRC. Aspirin/NSAID use appeared to lower risk of PTGS2-positive cancers, particularly among individuals with high levels of circulating MIC-1.

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Considerable evidence suggests that chronic inflammation plays an important role in the development of colorectal cancer (CRC). Individuals with long-standing colonic inflammation due to inflammatory bowel disease have an increased susceptibility to CRC (1). Furthermore, nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, are associated with a lower risk of CRC and improved clinical outcomes after diagnosis (2,3,4).

One proposed pathway through which inflammation promotes carcinogenesis is the overexpression of PTGS2 (prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2 [COX-2]) (5,6). PTGS2-positive colorectal tumors appear to respond more favorably to aspirin and NSAIDs than PTGS2-negative tumors (2).

The circulating inflammatory cytokine growth differentiation factor 15 (GDF15), also known as macrophage inhibitory cytokine-1 (MIC-1), may be an important mediator in the systemic inflammatory response (7). MIC-1 levels rise in the circulation in response to injury or inflammation (8). Elevated levels of MIC-1 have been previously associated with an increased risk of diseases hypothesized to result from chronic inflammation, such as atherosclerosis and inflammatory arthritis (8,9). MIC-1 has also been linked to the development of cancers, including those of the prostate, thyroid, pancreas, and colon (7,10,11). Recently, within the Polyp Prevention Trial, MIC-1 levels were associated with an increased risk of recurrent adenoma among patients with a history of prior adenoma (12). Experimental evidence also suggests that MIC-1, as a member of the human transforming growth factor-β (TGFβ1) superfamily (8), may play a specific role in carcinogenesis (7,13–15).

Although we and others have examined the relationship between other inflammatory markers—including C-reactive protein (CRP), interleukin 6 (IL-6), soluble tumor necrosis factor receptor 2 (also known as TNFRSF1B), and adiponectin—and the risk of CRC, prospective data relating MIC-1 levels and incident CRC are limited

(16,17). Therefore, we examined the association between prediagnostic levels of MIC-1 risk of CRC in a prospective, nested, case–control study of men and women enrolled in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). We also explored the association between MIC-1 levels and CRC according to the expression status of intratumoral PTGS2.

Methods

Study Populations

Data for this analysis were drawn from two ongoing cohorts, the NHS and the HPFS. The NHS began in 1976 among 121 700 US female registered nurses aged 30 to 55 years at enrollment. The HPFS began in 1986 among 51 529 US male podiatrists, dentists, osteopathic physicians, veterinarians, pharmacists, and optometrists aged 40 to 75 years at enrollment. In both cohorts, participants have biennially returned questionnaires with greater than 90% follow-up to provide information about lifestyle and dietary factors, medication use, and diagnoses of CRC and other diseases. The Committee on Human Studies of the Brigham and Women's Hospital and Harvard University approved this study. Completion of self-administered questionnaires was considered to imply informed consent.

Blood Collection

We collected blood samples by mailing phlebotomy kits to participants. From 1989 to 1990, 32 826 NHS participants, and from 1993 to 1995, 18 225 HPFS participants returned blood samples on ice packs by overnight courier. Upon receipt, blood samples were immediately centrifuged, aliquoted into plasma, and stored in continuously monitored liquid nitrogen freezers (–130°C or colder). More than 95% of the blood samples arrived in our laboratory within 26 hours of phlebotomy. Further details regarding blood collection, transportation of samples, and storage within these two cohorts has been previously described (18,19).

Selection of Colorectal Cancer Case Patients and Control Participants

Eligible men and women for this study provided a blood specimen, completed the baseline questionnaire, and did not have a history of inflammatory bowel disease or cancer (except nonmelanoma skin cancer). Incident cases of CRC through 2008 follow-up were initially self-reported and then confirmed with hospital records or pathology reports. We identified deaths through next-of-kin and the National Death Index. For all deaths, we sought information to determine the cause, including death certificates and medical records. A study physician, blinded to exposure information, reviewed all records to confirm cases, as well as to extract data on histological type, anatomic location, and stage of the cancer. Then, using risk-set sampling, we randomly selected up to two control subjects from among participants who had not developed cancer by the age of the corresponding case patient, matching on sex (cohort), year of birth, and month of blood draw. We excluded 33 case patients and their matched control subjects that failed laboratory assays. After these exclusions, we included 618 incident CRC case patients and 950 control subjects for this analysis.

Laboratory Assays

In a core laboratory facility, personnel blinded to case–control status used sandwich enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN) to measure MIC-1 (GDF15) levels in the archived prediagnostic plasma specimens. In HPFS, we assayed case patients and control subjects identified from 1993 to 2008 in a single batch. In NHS, we assayed case patients and control subjects identified from 1990 to 2004 follow-up in one batch and case patients and control subjects identified from 2006 to 2008 in a second batch. All samples from case patients and each of their matched control subjects were analyzed in the same batch. Based on quality controls randomly interspersed among the case–control samples, the coefficients of variation were 9.0% in HPFS, 7.0% in NHS (1990–2004), and 11.0% in NHS (2006–2008). In these cohorts, we have previously described our measurements of other inflammatory biomarkers, including high-sensitivity CRP, IL-6, TNFRSF1B, and adiponectin (16,20).

Assessment of PTGS2 (COX-2) Expression

Among CRC case patients in NHS and HPFS with available tumor specimens, we conducted immunohistochemical staining for PTGS2 as previously described (16). A pathologist (S. Ogino) who was unaware of any data concerning the participants scored the tumor epithelial PTGS2 expression according to the intensity of staining compared with surrounding normal epithelium using a standardized grading scheme. As a result, tumors with moderate or strong staining were classified as PTGS2 positive, whereas tumors with weak or absent staining were classified as PTGS2 negative. For an agreement study, a random selection of 124 case patients was examined by a second observer (T. Morikawa) unaware of other data. The concordance between the two observers ($P < 0.001$) was a kappa of 0.69, indicating substantial agreement.

Statistical Analysis

We calculated means, medians, and proportions for baseline characteristics of study participants and then used parametric or nonparametric methods (ie, paired *t* test, χ^2, or Wilcoxon signed rank tests) to compare case patients and control subjects. We calculated Spearman coefficients to estimate correlations between mean levels of inflammatory biomarkers, including MIC-1, and lifestyle factors among control subjects.

To examine the effect of MIC-1 levels on the risk of CRC, we categorized MIC-1 into quintiles based upon the distribution among the control subjects within each cohort. We estimated relative risks (RRs) for CRC with 95% confidence intervals (CIs) using logistic regression. We also tested for trend by assigning each participant to the median of their quintile and entering these values as continuous terms in the logistic regression model. Our results were similar when we used conditional and unconditional (adjusting for matching factors) logistic regression; thus, we present the results of unconditional logistic regression models. To determine if there was a nonlinear association between MIC-1 and CRC risk, we fit a restricted cubic spline function, which has been described elsewhere (16).

In multivariable analysis, we adjusted for matching factors as well as potential confounders, including pack-years of smoking; race; alcohol consumption; body mass index (BMI); physical activity (in metabolic equivalent [MET]–hours/week); family history of CRC; regular use of aspirin or NSAIDs; regular use of multivitamins; postmenopausal hormone use (in women); red meat intake;

energy-adjusted intake of calcium and folate; previous diagnosis of polyp; history of endoscopic screening; and the inflammatory markers CRP, IL-6, TNFRSF1B, and adiponectin (as continuous measures). We also conducted stratified analyses by various CRC risk factors. To test for multiplicative interaction between stratification factors and biomarkers, we included cross-product terms between stratification factors and the continuous value of MIC-1 in our models.

To examine potential heterogeneity in the associations between MIC-1 and CRC according to cancer subsite (colon or rectum) and PTGS2 tumor status, we used a polytomous logistic regression model in which the association with MIC-1 was allowed to vary between the case patient groups. To calculate the statistical significance between case patient groups, we performed a likelihood ratio test comparing the model described above to a model in which the MIC-1 quintile variables were held constant. Finally, we tested for trend including MIC-1 as a continuous variable in the PTGS2 logistic regression models.

We used SAS version 9.2 (SAS Institute, Cary, NC) for all analyses with the exception of polytomous logistic regression models for which we used Stata version 11.0 (StataCorp, College Station, TX). All statistical tests were two-sided, and P less than .05 was considered statistically significant.

Results

Baseline Cohort Characteristics

Table 1 shows the baseline characteristics of the 618 CRC case patients and the 950 matched control subjects at the time of blood draw. CRC case patients were more likely to have a higher BMI, whereas control subjects were more likely to regularly use multivitamins and aspirin/NSAIDs and to consume more folate. Supplementary Figure 1 (available online) illustrates the distribution of MIC-1 levels among HPFS and NHS cases and controls. The Spearman correlation coefficients between MIC-1, age, BMI, MET task score, and other inflammatory markers within sex-specific strata are shown in Supplementary Tables 1 and 2 (available online). Among both men and women, MIC-1 directly correlated with age and waist-to-hip ratio as well as plasma levels of TNFRSF1B, IL-6, CRP, and total adiponectin.

Plasma MIC-1 and Risk of Colorectal Cancer

We examined the association of MIC-1 with risk of CRC according to quintile categories determined by the distribution among control subjects (Table 2). Compared with men and women with levels of plasma MIC-1 in the lowest quintile (Q1), the multivariable relative risks for CRC were 1.36 (95% CI = 0.95 to 1.95) for those with MIC-1 levels in the second quintile, 1.68 (95% CI = 1.16 to 2.43) for those with levels in the third quintile, 1.88 (95% CI = 1.27 to 2.77) for those with levels in the fourth quintile, and 1.93 (95% CI = 1.27 to 2.94) for those with levels in the highest quintile after adjusting for known risk factors for CRC and plasma CRP, IL-6, TNFRSF1B, and adiponectin ($P_{\text{linear trend}}$ = .004). Although the association between MIC-1 and CRC appeared to be more evident among men compared with women, we did not observe any statistically significant heterogeneity ($P_{\text{heterogeneity}}$ = .13). We explored the shape of the relationship between MIC-1 levels and the risk of CRC using restricted cubic splines. A test for overall significance of the curve was statistically significant (P = .02) (Figure 1).

Table 1. Baseline characteristics of case patients and control subjects at the time of blood draw*

Characteristic	NHS		HPFS	
	Case patients (n = 344)	Control subjects (n = 419)	Case patients (n = 274)	Control subjects (n = 531)
Age at blood draw, y, mean (SD)	58.9 (6.72)	58.9 (6.77)	65.8 (8.30)	65.8 (8.27)
Race				
White, %	97.4	99.1	93.8	94.2
Other, %	2.60	0.90	6.2	5.8
Smoking status				
Current or past, %	57.3	56.1	55.5	51.3
Never, %	42.7	43.9	45.5	48.7
Postmenopause, %	88.5	88.4	—	—
Post-menopausal hormone use				
Never, %	48.8	51.3		
Past, %	20.1	14.3	—	—
Current, %	31.1	34.4		
Regular use of aspirin or NSAIDs, ≥2 tablets/wk, %	45.9	53	48.9	54.1
Current use of multivitamins, %	33.1	35	47.5	51.7
Colorectal cancer in a parent or sibling, %	13.1	13	19.7	13.7
History of screening prior to diagnosis, %	11.9	12.7	56.2	67.3
History of colon polyp, %	3.49	3.58	13.9	15.2
Body mass index, kg/m^2, mean (SD)	25.9 (4.94)	25.5 (4.92)	26.2 (3.05)	25.3 (2.71)
Physical activity, METs, mean (SD)	15.4 (16.1)	16.8 (20.3)	32.1 (29.8)	34.6 (34.9)
Alcohol intake, g/d, mean (SD)	5.63 (9.52)	5.61 (8.90)	12.1 (14.7)	11.9 (14.6)
Calcium, mg/d, mean (SD)	976 (406)	1025 (407)	917 (385)	928 (343)
Folate, mg/d, mean (SD)	398 (180)	422 (187)	494 (208)	520 (228)
Beef as main dish, servings/d, mean (SD)	0.30 (0.17)	0.29 (0.16)	0.27 (0.21)	0.26 (0.18)
MIC-1, pg/mL, median (IQR)	754 (603–930)	748 (567–959)	900 (671–1132)	825 (610–1061)

* HPFS = Health Professionals Follow-up Study (men only); IQR = interquartile range; METs = metabolic equivalent task score hours per week; MIC-1 = macrophage inhibitory cytokine-1; NHS = Nurses' Health Study; NSAID = nonsteroidal anti-inflammatory drugs; SD = standard deviation.

Table 2. Relative risk of colorectal cancer according to plasma levels of MIC-1*

Analysis	Quintile of plasma MIC-1†					P_{trend}‡
	1	2	3	4	5	
Combined (n = 1568)						
No. of case patients/control subjects	93/191	116/190	133/191	138/189	138/189	
Age-adjusted RR (95% CI)§	1.00 (referent)	1.33 (0.94 to 1.88)	1.57 (1.09 to2.24)	1.74 (1.20 to 2.53)	1.80 (1.22to 2.64)	.005
MV-adjusted RR (95% CI)‖	1.00 (referent)	1.37 (0.96 to 1.94)	1.59 (1.10 to 2.28)	1.78 (1.22 to 2.60)	1.80 (1.22 to 2.67)	.007
MV-adjusted RR (95% CI)¶	1.00 (referent)	1.36 (0.95 to 1.95)	1.68 (1.16 to 2.43)	1.88 (1.27 to 2.77)	1.93 (1.27 to 2.94)	.004
Men (n = 805)						
Median, pg/mL	501	649	825	1000	1286	
Cutpoints, pg/mL	0–575	575–747	747–890	890–1109	1109–3266	
No. of case patients/control subjects	42/107	51/106	44/107	64/106	73/106	
Age-adjusted RR (95% CI)§	1.00 (referent)	1.36 (0.82 to 2.25)	1.29 (0.75 to 2.24)	2.00 (1.16 to 3.47)	2.40 (1.36 to 4.23)	.001
MV-adjusted RR (95% CI)‖	1.00 (referent)	1.39 (0.83 to 2.35)	1.19 (0.67 to 2.09)	2.01 (1.14 to 3.54)	2.28 (1.27 to 4.10)	.003
MV-adjusted RR (95% CI)¶	1.00 (referent)	1.44 (0.85 to 2.43)	1.26 (0.71 to 2.23)	2.21 (1.24 to 3.95)	2.76 (1.48 to 5.17)	.0006
Women (n = 763)						
Median, pg/mL	483	599	749	926	1218	
Cutpoints, pg/mL	0–544	544–664	664–818	818–1014	1014–3256	
No. of case patients/control subjects	51/84	65/84	89/84	74/84	65/83	
Age-adjusted RR (95% CI)§	1.00 (referent)	1.34 (0.82 to 2.17)	1.84 (1.13 to 2.99)	1.57 (0.94 to 2.62)	1.39 (0.82 to 2.35)	.46
MV-adjusted RR (95% CI)‖	1.00 (referent)	1.34 (0.82 to 2.18)	1.88 (1.15 to 3.07)	1.56 (0.93 to 2.63)	1.41 (0.82 to 2.41)	.46
MV-adjusted RR (95% CI)¶	1.oo (referent)	1.25 (0.75 to 2.08)	2.02 (1.21 to 3.38)	1.58 (0.91 to 2.73)	1.29 (0.71 to 2.32)	.66

* CI = confidence interval; MIC-1 = macrophage inhibitory cytokine-1; MV = multivariable; RR = relative risk.

† Quintiles of plasma MIC-1 were calculated separately within each cohort based on the distribution in the controls. For the combined analysis, we assigned participants to quintile categories based upon cohort-specific cutpoints. Two-sided $P_{interaction}$ for the association between MIC-1 and colorectal cancer among men compared with women was .13.

‡ Tests for linear trend were conducted using the median values for each quintile in a logistic regression model. *P* values are two-sided

§ Age-adjusted models include adjustment for matching factors (age at blood draw, date of blood draw, race, and sex).

‖ Multivariable models were adjusted for age at blood draw, date of blood draw, race, sex, body mass index, physical activity (metabolic equivalent task score hours per week), current or past smoking (yes or no), prior/current use of postmenopausal hormones (yes or no), prior history of screening (yes or no), previous occurrence of adenoma, colorectal cancer in parent or sibling, regular use of multivitamins, regular use of aspirin or nonsteroidal anti-inflammatory drugs (≥2 tablets per week), energy-adjusted intake (including supplements) of calcium and folate, servings of red meat as a main dish, and alcohol consumption.

¶ Multivariable model additionally adjusted for the plasma inflammatory markers C-reactive protein, soluble tumor necrosis factor receptor 2, interleukin 6, and total adiponectin as continuous measures, as well as age at blood draw, date of blood draw, race, sex, body mass index, physical activity (metabolic equivalent task score hours per week), current or past smoking (yes or no), prior/current use of postmenopausal hormones (yes or no), prior history of screening (yes or no), previous occurrence of adenoma, colorectal cancer in parent or sibling, regular use of multivitamins, regular use of aspirin or nonsteroidal anti-inflammatory drugs (≥2 tablets per week), energy-adjusted intake (including supplements) of calcium and folate, servings of red meat as a main dish, and alcohol consumption.

To assess whether there was effect modification by selected lifestyle risk factors for CRC, we performed stratified analyses of MIC-1 according to age at time of blood collection, smoking, BMI, family history of CRC, regular aspirin/NSAID use, history of polyps, and history of endoscopic screening (Table 3). No statistically significant interactions were identified between MIC-1 levels and these CRC risk factors. We also did not observe material differences between MIC-1 and risk of cancers of the colon compared with rectum.

To address the possibility that occult or subclinical CRC could influence levels of MIC-1, we excluded case patients diagnosed up to 4 years after blood draw from our analysis and observed similar results (multivariable RR for CRC = 1.65; 95% CI = 1.10 to 2.47), comparing extreme quintiles of MIC-1. In stratified analysis of MIC-1 and risk of CRC, we also found similar results when comparing case patients diagnosed within 4 years of blood draw ($P_{linear\ trend}$ = .03) and case patients diagnosed 4 years after blood draw ($P_{linear\ trend}$ = .02). Finally, because cardiovascular disease and other cancers have been associated with higher levels of MIC-1, we also conducted analyses excluding case patients with cardiovascular disease or any cancer diagnosed between the time of enrollment and within 2 years after blood draw and observed similar results (multivariable RR for CRC = 1.65; 95% CI = 1.06 to 2.58 comparing extreme quintiles of MIC-1; $P_{linear\ trend}$ = .04).

Association Between MIC-1 Levels and CRC According to PTGS2 Status

In an exploratory analysis, we considered the possibility that MIC-1 may be differentially associated with risk of CRC according to intratumoral PTGS2 expression among the 245 case patients for whom there was available tumor tissue for analysis (Supplementary Table 3, available online). Compared with men and women with levels of plasma MIC-1 in the lowest quintile, the multivariable relative risks for PTGS2-positive CRC were 1.65 (95% CI = 0.93 to 2.93) for those with MIC-1 levels in the second quintile, 1.48 (95% CI = 0.79 to 2.77) for those with levels in the third quintile, 1.92 (95% CI = 1.02 to 3.64) for those with levels in the fourth quintile, and 1.58 (95% CI = 0.78 to 3.20) for those with levels in the highest quintile ($P_{linear\ trend}$ = .18). In contrast, the corresponding relative risks for PTGS2-negative CRC were 1.10 (95% CI = 0.55 to 2.17) for those with MIC-1 levels in the second quintile, 0.66 (95% CI = 0.30 to 1.45) for those with levels in the third quintile, 0.61 (95% CI = 0.27 to 1.42) for those with levels in the fourth quintile, and 0.80 (95% CI = 0.34 to 1.89) for those with levels in the highest quintile ($P_{linear\ trend}$ = .24). Nonetheless, a formal test of heterogeneity in the association of MIC-1 and CRC according to PTGS2 status was not statistically significant ($P_{heterogeneity}$ = .22).

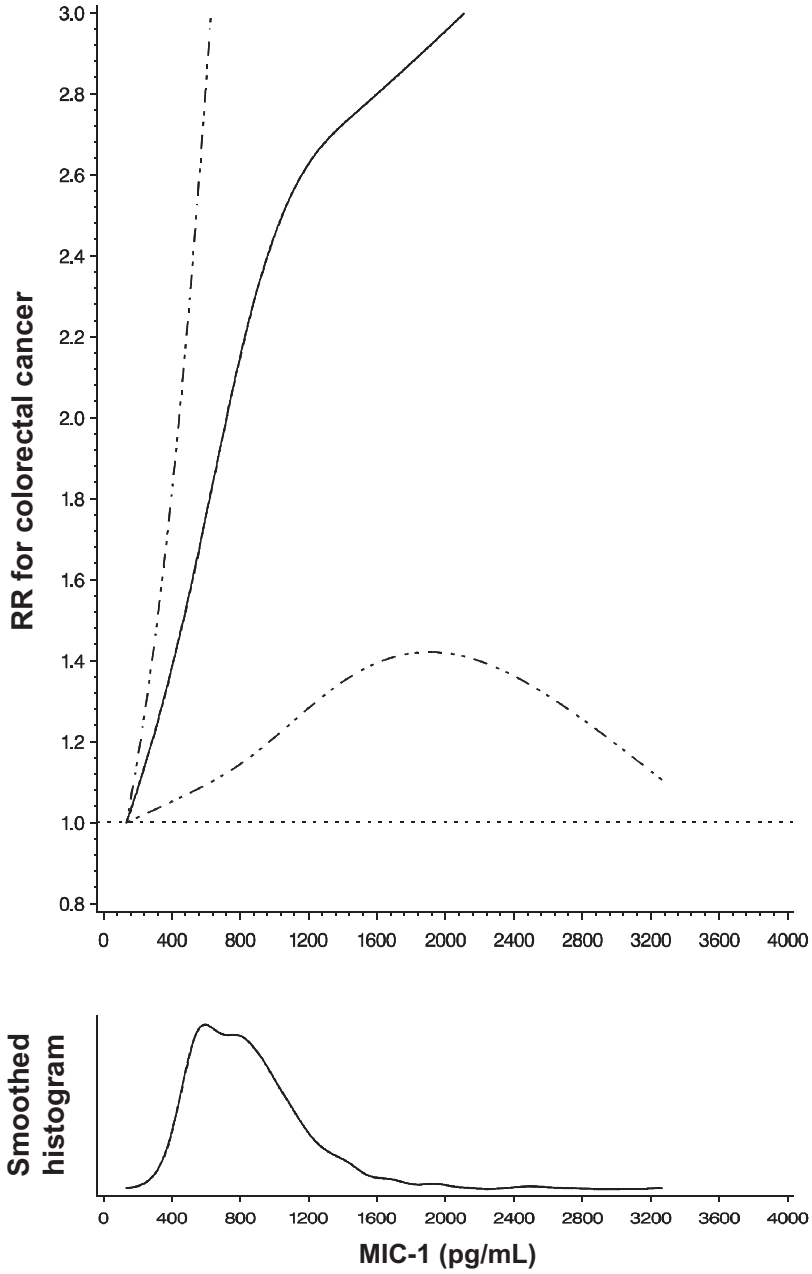

Figure 1. Restricted cubic spline plot for macrophage inhibitory cytokine 1 (MIC-1) and risk of colorectal cancer. Relative risk (RR) of colorectal cancer is plotted accordingly to serum MIC-1 level (pg/mL). **Hatched lines** represent 95% confidence intervalss. Spline was adjusted for age at blood draw, date of blood draw, race, sex, body mass index, physical activity (metabolic equivalent task score hours per week), current or past smoking (yes or no), prior/current use of postmenopausal hormones (yes or no), prior history of screening (yes or no), previous occurrence of adenoma, colorectal cancer in parent or sibling, regular use of multivitamins, regular use of aspirin or nonsteroidal anti-inflammatory drugs (≥2 tablets per week), energy-adjusted intake (including supplements) of calcium and folate, servings of red meat as a main dish, and alcohol consumption. A test for overall significance of the curve was statistically significant (P = .02). The test for curvature (ie, nonlinear relation) was 0.12. Test for linear relation was 0.01. All P values listed here are two-sided. A smoothed histogram below the plot shows the distribution of MIC-1 among case patients and control subjects in both cohorts.

Association of Aspirin/NSAID Use and Risk of PTGS2-positive CRC According to MIC-1 Levels

In an exploratory analysis, we examined whether aspirin/NSAID use may be differentially associated with risk of PTGS2-positive tumors according to baseline levels of MIC-1 (Supplementary Table 4, available online). Among individuals with high plasma MIC-1 levels (quintiles 2–5), compared with nonuse, regular use of aspirin and NSAIDs was associated with a lower risk of PTGS2-positive CRC (multivariable RR = 0.60; 95% CI = 0.41 to 0.88) but not PTGS2-negative CRC (multivariable RR = 1.21; 95% CI = 0.71 to 2.07). In contrast, among individuals with low MIC-1 levels (quintile 1), aspirin and NSAID use was not associated with a lower risk of PTGS2-positive CRC (multivariable RR = 0.57; 95% CI = 0.21 to 1.54) or PTGS2-negative CRC (multivariable RR = 1.41; 95% CI = 0.47 to 4.23).

Table 3. Relative risk of colorectal cancer according to plasma MIC-1 within selected subgroups*

Subgroup	Analysis	Quintiles of plasma MIC-1					P_{trend}†	$P_{interaction}$‡
		1	2	3	4	5		
Age <65 y (n = 329)	No. of Cases/Controls	36/88	33/69	16/30	16/22	11/8		.32
	MV RR (95% CI)	1.00 (referent)	1.15 (0.60 to 2.17)	1.11 (0.48 to 2.57)	2.16 (0.88 to 5.29)	4.20 (1.31 to 13.5)	.01	
Age ≥ 65 y (n = 1239)	No. of Cases/Controls	57/103	83/121	117/161	122/167	127/181		
	MV RR (95% CI)	1.00 (referent)	1.34 (0.85 to 2.11)	1.67 (1.08 to 2.61)	1.79 (1.13 to 2.84)	1.73 (1.07 to 2.81)	.07	
No history of smoking	No. of Cases/Controls	48/107	48/90	67/91	53/83	53/72		.18
(n = 676)	MV RR (95% CI)	1.00 (referent)	1.19 (0.70 to 2.03)	2.03 (1.17 to 3.49)	2.08 (1.13 to3.81)	2.39 (1.25 to 4.57)	.007	
Past or current smoker	No. of Cases/Controls	45/84	68/100	66/100	85/106	85/117		
(n = 852)	MV RR (95% CI)	1.00 (referent)	1.45 (0.88 to 2.39)	1.38 (0.82 to 2.34)	1.81 (1.07 to 3.09)	1.61 (0.90 to 2.86)	.17	
Nonuser of aspirin or	No. of Cases/Controls	60/114	59/92	73/82	68/79	66/74		.58
NSAIDs (n = 767)	MV RR (95% CI)	1.00 (referent)	1.31 (0.80 to 2.12)	1.94 (1.17 to 3.22)	2.04 (1.19 to 3.51)	2.45 (1.34 to 4.45)	.003	
Regular user of aspirin or	No. of Cases/Controls	33/77	57/98	60/109	70/110	72/115		
NSAID (n = 801)	MV RR (95% CI)	1.00 (referent)	1.48 (0.85 to 2.57)	1.54 (0.87to 2.73)	1.75 (0.97 to 3.14)	1.78 (0.96 to 3.31)	.15	
Low BMI§ (n = 695)	No. of Cases/Controls	35/88	46/89	54/86	61/100	55/81		.23
	MV RR (95% CI)	1.00 (referent)	1.25 (0.71 to 2.23)	1.62 (0.89 to 2.97)	1.96 (1.05 to 3.64)	1.99 (1.01 to 3.94)	.04	
High BMI‖ (n = 873)	No. of Cases/Controls	58/103	70/101	79/105	77/89	83/108		
	MV RR (95% CI)	1.00 (referent)	1.39 (0.87 to 2.22)	1.62 (1.00 to 2.62)	1.91 (1.13 to 3.20)	1.90 (1.09 to 3.29)	.04	
No family history of	No. of Cases/Controls	79/169	103/168	112/163	114/171	111/154		.78
colorectal cancer (n=224)	MV RR (95% CI)	1.00 (referent)	1.44 (0.98 to 2.12)	1.77 (1.18 to 2.65)	1.79 (1.17 to 2.73)	2.23 (1.41 to 3.54)	.002	
Family history of colorectal	No. of Cases/Controls	14/22	13/22	21/28	24/18	27/35		
cancer (n=1344)	MV RR (95% CI)	1.00 (referent)	0.96 (0.34 to 2.72)	1.41 (0.50 to 4.00)	2.44 (0.80 to 7.55)	1.01 (0.33 to 3.04)	.97	
No history of colon polyps	No. of Cases/Controls	90/178	108/175	124/165	121/167	125/169		.17
(n = 1422)	MV RR (95% CI)	1.00 (referent)	1.28 (0.89 to 1.86)	1.66 (1.14 to 2.47)	1.67 (1.11 to 2.51)	1.81 (1.17 to 2.81)	.02	
History of colon polyps	No. of Cases/Controls	3/13	8/15	9/26	17/22	13/20		
(n = 146)	MV RR (95% CI)	1.00 (referent)	3.18 (0.52 to 2.22)	1.68 (0.29 to 9.55)	6.06 (1.04 to 35.3)	3.78 (0.55 to 26.1)	.19	
No history of prior screen-	No. of Cases/Controls	68/121	86/103	99/105	90/102	80/105		.09
ing (n = 959)	MV RR (95% CI)	1.00 (referent)	1.45 (0.94 to 2.23)	1.73 (1.11 to 2.71)	1.58 (0.98 to 2.56)	1.30 (0.77 to 2.17)	.72	
History of prior screening	No. of Cases/Controls	25/70	30/87	34/86	48/87	58/84		
(n = 609)	MV RR (95% CI)	1.00 (referent)	1.20 (0.61 to 2.35)	1.69 (0.84 to 3.39)	2.72 (1.34 to 5.52)	4.18 (1.95 to 8.93)	<.0001	
<4 years after blood draw	No. of cases/controls	15/41	22/31	32/44	32/56	42/47	.03	.98
(n = 360)	MV RR (95% CI)	1.00 (referent)	2.32 (0.93 to 5.76)	2.64 (1.08 to 6.47)	2.48 (0.98 to 6.30)	4.10 (1.48 to 11.3)		
≥4 years after blood draw	No. of cases/controls	78/150	94/159	101/147	106/133	96/142	.02	
(n = 1208)	MV RR (95% CI)	1.00 (referent)	1.28 (0.86 to 1.90)	1.57 (1.04 to 2.38)	1.90 (1.23 to 2.95)	1.70 (1.06 to 2.73)		

* BMI = body mass index; CI = confidence interval; MIC-1 = macrophage inhibitory cytokine-1; MV = multivariable; NSAIDs = nonsteroidal anti-inflammatory drugs; RR = relative risk.

† Tests for linear trend were conducted using the median values for each quintile in a logistic regression model. *P* values are two-sided.

‡ Tests for interaction were conducted using cross-product terms between stratification factors and the continuous value of MIC-1 in logistic regression models. *P* values are two-sided.

§ Low BMI defined as less than the median (<24.0 kg/m^2 for women and <25.0 kg/m^{2}for men).

‖ High BMI defined as greater than the median (≥24.0 kg/m^2 for women and ≥25.0 kg/m^2 for men).

¶ Family history of colorectal cancer includes at least one sibling or parent with colorectal cancer.

Discussion

In this prospective study, we observed that men and women in the highest quintile of MIC-1 had a nearly twofold higher risk of incident CRC. Results were similar in men and women and persisted after adjusting for other known lifestyle risk factors for CRC, as well as other plasma inflammatory biomarkers, including CRP, IL-6, TNFRSF1B, and adiponectin. The associations did not materially change according to subgroups defined by age, BMI, smoking status, regular use or nonuse of aspirin/NSAIDs, family history of CRC, history of polyps, and history of screening. Our results, which included a large number of case patients with incident CRC (n = 618), are consistent with and extend upon a prior smaller cross-sectional investigation demonstrating an association between MIC-1 and CRC (n = 58) (10). Similarly, in a recent secondary analysis of the Polyp Prevention Trial, elevated levels of MIC-1 were associated with the presence of adenoma and an increased risk of recurrent colorectal adenoma, which appeared particularly evident among users of aspirin/NSAIDs (12).

Our findings support the role of chronic inflammation in the development of CRC. Moreover, because MIC-1 was associated with risk of CRC even after accounting for other inflammatory markers, our data suggest that MIC-1 may be a more specific predictor of CRC than these other biomarkers. This is supported by experimental evidence demonstrating a specific mechanistic role for MIC-1 in tumor development. MIC-1 is a member of the human TGFβ1 superfamily (8). Like TGFβ1 itself, MIC-1 appears to have a pleiotropic effect, inhibiting neoplasia in early stages (eg, tumor initiation) but perhaps promoting progression in later stages (eg, metastasis) (21). In vitro, MIC-1 is activated by the p53 gene product and is upregulated by anticancer compounds, such as those in cruciferous vegetables (7). In animal models, MIC-1 gene expression suppresses the formation of azoxymethane-induced colonic tumors and mediates the chemopreventive effect of NSAIDs (7,13–15). Under some circumstances, however, MIC-1 may play a protumorigenic role. In mouse xenograft models, MIC-1 enhances tumor growth, stimulates cell proliferation, and promotes distant metastases (7). Furthermore, in humans, a cross-sectional study that included 224 case patients with prevalent CRC showed that MIC-1 levels were associated with increased tumor stage, presence of metastases, and earlier disease relapse (10). Nonetheless, regardless of its specific role in either inhibiting or stimulating carcinogenesis, our findings provide evidence that circulating levels of MIC-1 are elevated prediagnostically and have potential as a biomarker for CRC risk.

Although we had a limited number of case patients with available tissue for PTGS2 analysis, in exploratory analyses it appeared that the association between MIC-1 and CRC was more evident among PTGS2-positive tumors compared with PTGS2-negative tumors. Among the individuals with PTGS2-positive tumors and with elevated serum levels of MIC-1, we found evidence that participants taking aspirin/NSAIDs had a particularly lower risk of PTGS2-positive cancer. These findings provide additional mechanistic specificity to our results, suggesting that MIC-1 is primarily associated with cancers that arise through a proinflammatory milieu. Furthermore, they are consistent with findings in animal models that MIC-1 may mediate the antitumorigenic effect of aspirin/NSAID use. Similarly, aspirin/NSAID use reduced adenoma recurrence primarily among individuals with elevated MIC-1 levels in the Polyp Prevention Trial (12,13).

There are several strengths of our study, including its large size, prospective design, and high follow-up rate. By measuring MIC-1 levels before diagnosis of cancer, we minimized potential bias related to elevation of this marker by the cancer itself. Second, our matched control subjects were selected from the same cohort as the case patients, minimizing population stratification or selection bias. Third, our findings were consistent between two independent cohorts. Finally, we were able to examine associations after accounting for other inflammatory cytokines.

There are also limitations to this study. First, a single measurement of MIC-1 in relation to subsequent CRC risk may underestimate associations due to regression dilution bias (22). Second, because our participants were all health professionals, our study may not be generalizable to the greater US population.

In summary, our results demonstrate a higher risk of CRC associated with elevated prediagnostic MIC-1 (GDF15) levels, even after accounting for other CRC risk factors and inflammatory cytokines. These findings suggest that MIC-1 may be a specific marker for risk of CRC and provide additional evidence for the importance of chronic inflammation in the pathogenesis of CRC. Additional studies are needed to confirm these findings and further explore a mechanistic role for MIC-1 in the development and progression of CRC.

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