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Association Between Inflammatory Diet Pattern and Risk of Colorectal Carcinoma Subtypes Classified by Immune Responses to Tumor

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Abstract

Background & Aims—Dietary patterns affect systemic and local intestinal inflammation, which have been linked to colorectal carcinogenesis. Chronic inflammation can interfere with the adaptive immune response. We investigated whether the association of a diet that promotes intestinal inflammation with risk of colorectal carcinoma was stronger for tumors with lower lymphocytic reactions than tumors with higher lymphocytic reactions.

Methods—We collected data from the molecular pathological epidemiology databases of 2 prospective cohort studies: the Nurses' Health Study (since 1976) and the Health Professional Follow-up Study (since 1986). We used duplication-method time-varying Cox proportional cause-

specific hazards regression to assess the association of empirical dietary inflammatory pattern (EDIP) score (derived from food frequency questionnaire data) with colorectal carcinoma subtype. Foods that contribute to high EDIP scores include red and processed meats, refined grains, carbonated beverages, and some vegetables; foods that contribute to low EDIP scores include beer, wine, coffee, tea, yellow and leafy vegetables, and fruit juice. Colorectal tissue samples were analyzed histologically for patterns of lymphocytic reactions (Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction, and tumor-infiltrating lymphocytes).

Results—During follow up of 124,433 participants, we documented 1311 incident colon and rectal cancer cases with available tissue data. The association between the EDIP and colorectal cancer risk was significant (P_{trend} = .02), and varied with degree of peritumoral lymphocytic reaction ($P_{heterogeneity}$ < .001). Higher EDIP scores were associated with increased risk of colorectal cancer with an absent or low peritumoral lymphocytic reaction (highest vs lowest EDIP score quintile hazard ratio = 2.60; 95% CI, 1.60–4.23; P_{trend} < .001) but not risk of tumors with intermediate or high peritumoral lymphocytic reaction (P_{trend} > .80).

Conclusions—In a prospective cohort study, we associated inflammatory diets with a higher risk of colorectal cancer subtype that contains little or no peritumoral lymphocytic reaction. These findings suggest that diet-related inflammation might contribute to development of colorectal cancer, by suppressing the adaptive anti-tumor immune response.

Keywords

adaptive immune cells; BRAF; CpG island methylator phenotype; cyclooxygenase-2

INTRODUCTION

Accumulating evidence indicates that inflammation plays a critical role in colorectal carcinogenesis,[1] and that certain dietary components have demonstrable influence on systemic and gastrointestinal inflammatory status, consequently impacting colorectal carcinogenesis.[2–4] In particular, diets that induce inflammation (referred to as inflammatory diets) appear to exert their cancer-promoting potential though the effects of pro-inflammatory mediators, such as interleukin 1 (IL1), IL6, and TNF (tumor necrosis factor-α). These inflammatory mediators can act as pro-oncogenic factors through activation of downstream oncogenic signaling pathways (including PI3K / AKT / MTOR and MAPK / ERK signaling cascades), enhancing cell growth, proliferation and migration.[5–7] Epidemiological studies have shown that highly inflammatory diets are associated with higher risk of colorectal cancer, especially proximal colon cancer and tumors without lymph node metastasis, and that the association appears to be stronger in males than females.[2–4] We have developed an empirical dietary inflammatory pattern (EDIP) score based on eighteen food groups that correlate with concentrations of inflammatory plasma biomarkers in the Nurses' Health Study (NHS).[6] The EDIP score has been validated in two independent cohorts of men and women, the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study-II, respectively.[6]

It is widely recognized that immune response plays a critical role in the host's control or elimination of neoplastic cells.[8, 9] During the processes of inflammation and tumorigenesis, a neoplastic lesion recruits various innate and adaptive immune cells. These immune cells communicate with each other by means of direct contact or cytokine and chemokine production to control and shape tumor growth. The interactions of various immune and inflammatory cells in the tumor microenvironment likely influence balances of the opposing effects of tumor-promoting inflammation and antitumor immunity.[10] Due to the complex balance between inflammation and immune modulation in tumorigenesis, pro-inflammatory diets may exert different effects according to interactions of tumor and immune cells. Such a variable risk modification by tumor-immunity interactions has been shown in a recent study on aspirin use in relation to incidence of colorectal cancer subtypes classified by tumor-infiltrating lymphocytes (TIL). Specifically, aspirin use has been associated with lower risk of colorectal cancer lacking abundant TIL.[11] Considering these findings, we hypothesized that the association of pro-inflammatory dietary patterns with colorectal cancer risk might be stronger for tumors that lacked intense lymphocytic reaction than for tumors with robust lymphocytic reaction.

To test this hypothesis, we utilized the database of the NHS and HPFS cohorts, and prospectively examined EDIP scores in relation to incidence of colorectal cancer subtypes classified by the patterns and degrees of lymphocytic reaction.

METHODS

Study Population

The study was based on participants in two ongoing prospective cohort studies, the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The NHS recruited 121,701 registered female nurses aged 30 to 55 years at baseline in 1976, and the HPFS enrolled 51,529 male health professionals aged from 40 to 75 years at baseline in 1986 in the United States.[12] In both cohorts, questionnaires were sent at baseline and every two years thereafter to collect and update demographic, lifestyle, medical, and other health-related information. Validated food frequency questionnaires were administrated in 1980, 1984 and 1986 and every 4 years thereafter in the NHS, and in 1986 and every 4 years thereafter in the HPFS to collect dietary data. We followed participants from the date of return of the baseline questionnaire through June 30th, 2012 in the NHS or January 31st, 2012 in the HPFS. We obtained written informed consent from all participants. This study was approved by Human Subjects Committees at Harvard T.H. Chan School of Public Health and Brigham and Women’s Hospital.

Assessment of Empirical Dietary Inflammatory Pattern (EDIP) Scores and Other Covariates

The development of the EDIP score has been previously described.[6] The goal was to empirically create a score for overall inflammatory potential of whole diets defined using food groups. The investigators entered 39 pre-defined food groups in reduced rank regression models followed by stepwise linear regression analyses to identify a dietary pattern most predictive of three plasma inflammatory biomarkers, IL6, CRP (C-reactive protein) and TNFRSF1B (TNFα-receptor 2).[13] The EDIP score is the weighted sum of 18

food groups, with higher (more positive) scores indicating pro-inflammatory diets and lower (more negative) scores indicating anti-inflammatory diets.[6] The detailed composition of the EDIP food group components is listed in Supplementary Table 1 and the formula used to compute EDIP scores is listed in Supplementary Methods in the Supplement. The validity of the EDIP score has been evaluated in two independent U.S.-based cohorts of women and men.[6] We calculated EDIP score for each participant based on food frequency questionnaire data at each questionnaire cycle. Since EDIP scores varied significantly between 1980 and thereafter in the NHS, we set 1984 as the study baseline for the NHS. The cumulative average EDIP score at each questionnaire cycle was further computed by averaging EDIP scores at all prior cycles up to the then-latest questionnaire cycle, to best represent habitual long-term dietary intake and reduce within-person variation. Participants were categorized into quintiles or quartiles using cohort-specific cut-off points of cumulative average EDIP scores at each time interval. Information on lifestyles, including smoking, physical activity, total energy intake, alcohol intake, multivitamin use, endoscopy status, regular aspirin use, family history of colorectal cancer, weight, height, and postmenopausal hormone use (only for women), was assessed using biennial questionnaires in both cohorts as previously described.[14, 15]

Ascertainment of Colorectal Cancer Cases

Incident colorectal cancer cases were identified using biennial questionnaires. The time (in month) until colorectal cancer diagnosis was measured from the date of the questionnaire return at the study baseline, which was 1984 for the Nurses' Health Study and 1986 for the Health Professional Follow-up Study. Lethal unreported colorectal cancer cases were identified through the National Death Index and next of kin. Diagnosis of colon or rectal carcinoma in participants was verified through medical record review in all cases included in this study.

Analyses of Histopathologic Lymphocytic Reaction and Tumor Characteristics

Paraffin-embedded archival tumor tissue blocks of confirmed colorectal cancer cases were collected from hospitals where the patients underwent tumor resection. A pathologist (S.O.) evaluated hematoxylin and eosin stained tissue sections and scored (as absent/low, intermediate, or high) for four histopathological patterns of lymphocytic reaction, namely Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction and tumor-infiltrating lymphocytes (TIL) (Supplementary Figure 1).[16] Of the 1311 tumors, 5 or more sections of each tumor were evaluated in 22 cases (2%), 4 sections in 57 cases (4%), 3 sections in 318 cases (24%), 2 sections in 317 cases (24%), and 1 section in 597 cases (46%). A subset of cases was re-examined by a second pathologist to ensure good concordance of histopathologic features as previously described.[16]

We extracted DNA from tumor and normal tissue and assessed for microsatellite instability (MSI),[17] CpG island methylator phenotype (CIMP),[18,19] *BRAF* mutation,[19] PTGS2 (cyclooxygenase-2) expression[20] as previously described. Further detail on the assessments of tumor immunity and molecular alterations is provided in Supplementary Methods.

Statistical Analysis

Participants who died of causes other than colorectal cancer, and those who were free of colorectal cancer at the end of follow-up were censored. In addition, colorectal cancer cases with unknown immune status were censored at the time of diagnosis. For each participant, we calculated follow-up time (in month) from the date of the questionnaire return at the study baseline until the date of death, colorectal cancer diagnosis, or end of follow-up, whichever came first. We used duplication-method time-varying Cox proportional cause-specific hazards regression analysis weighted by inverse probabilities for competing risks data[21, 22] to assess the associations of EDIP scores with risks of colorectal cancer subtypes classified by the degrees of lymphocytic reaction. Testing for trend across quintiles of EDIP scores was performed using the median value of each quintile group in the Cox regression models. To examine the heterogeneity in the associations with various colorectal cancer subtypes, we used a Wald test to assess the null hypothesis that the association with EDIP is equal for all subtypes. The primary hypothesis testing was set as a heterogeneity test (with its significance measure being $P_{\text{heterogeneity}}$) on the associations of EDIP scores with differential cancer subtypes classified by four different lymphocytic reaction markers.[22, 23] Hence, we adjusted the alpha level to 0.01 (≈ 0.05/4) by Bonferroni correction. All other analyses including evaluation of individual hazard ratios (HRs) represent secondary analyses. Inverse probability weighting (IPW) was used to reduce bias from potentially varied tumor tissue data availability, by calculation of the predictive probability of observing a specific immune marker for each case using multivariable logistic regression, which initially included sex, tumor stage, tumor location, age at diagnosis, year of diagnosis, body mass index, physical activity, total energy intake, multivitamin use, regular aspirin use, pack-years of smoking, family history of colorectal cancer, total alcohol intake and history of endoscopy. A backward elimination with a threshold P value of 0.1 was used to select variables for the final model. The weight of each case was set as one divided by the probability (the "inverse probability") of availability of specific tumor marker data, while it was set as 1 for non-cases and cases without data on the corresponding tissue marker in the weighted Cox regression models. Cox regression analyses were based on the counting process data structure and were stratified by age in months, calendar year of the questionnaire cycle and sex (in the combined analyses). We used the time-dependent Cox regression model to get immediate effects of pro-inflammatory diets on the risk of developing different subtypes of colorectal cancer according to lymphocytic reaction by controlling for the recent past potential confounders.[24] Multivariable-adjusted Cox regression models were adjusted for time-varying covariates (most of which were updated every 2 years), including family history of colorectal cancer, history of endoscopy, multivitamin use, regular aspirin use, pack-years of smoking, physical activity, total energy intake, alcohol intake (and postmenopausal hormone use only in the NHS). All multi-categorical covariates were treated as nominal variables. To validate our results, we conducted multiple sensitivity analyses. Given that overweight/obesity - a state of low-grade chronic inflammation - has been shown to play both a mediating and confounding role in associations between dietary inflammation potential and inflammation markers,[6] we further adjusted for body mass index as a sensitivity analysis. We further detected the association between quartiles of EDIP scores and risk of colorectal cancer subtypes classified by lymphocytic reaction. Because the time-dependent exposure (the EDIP score) and right

censoring of death from causes other than colorectal cancer might be informed by time-dependent covariates, we further considered marginal structural Cox proportional hazards models[25, 26] as a sensitivity analysis to detect the association between EDIP scores and risk of colorectal cancer subtypes according to immunity markers. We used the product of the stabilized inverse probability weights (IPWs) as the weights in the marginal structure model.[25,26] Stabilized IPWs were estimated by combining the stabilized inverse probability of treatment weights (IPTWs) and stabilized inverse probability of censoring weights (IPCWs).[25,26] SAS 9.4 (SAS Institute Inc, Cary, North Carolina, USA) was used for all statistical analyses. All statistical tests were two-sided.

RESULTS

Characteristics of Study Participants

The baseline characteristics of study participants are presented in Table 1 and Supplementary Table 2. In the Nurses' Health Study (NHS), we excluded 35,955 women without diet data in 1984, 315 participants with ulcerative colitis, 188 participants without birth dates, 6340 participants with any cancer diagnosed before 1984, and 1923 participants who died before 1984. In the Health Professionals Follow-up Study (HPFS), we excluded 1508 men without diet data in 1986, 509 participants with ulcerative colitis, 38 participants without birth dates, 2048 participants with any cancer diagnosed before 1986, and 12 participants who died in 1986. In total, 124,433 women and men were included into the current analyses. During 2,998,258 person-years of follow-up, we documented 1311 colorectal cancer cases with at least one tissue lymphocytic marker available. We did not observe evidence of a violation of the proportionality of hazards assumption on the basis of interaction terms between empirical dietary inflammatory pattern (EDIP) scores and follow-up time ($P = .60$). Excluding the colorectal cancer subtype with absent/low peritumoral lymphocytic reaction ($P_{heterogeneity} = .02$), we did not observe significant heterogeneity between cohorts for the associations of EDIP scores with risk of any other colorectal cancer subtypes. In order to increase statistical power, we combined the NHS and HPFS data to perform pooled analyses stratified by age in months, calendar year of the questionnaire cycle and sex.

EDIP and Risks of Colorectal Cancer Subtypes Classified by Lymphocytic Reaction to Tumor

We analyzed the associations between EDIP scores and incidence of overall colorectal cancer. There were statistical trends toward the associations of higher EDIP scores with an increased risk of colorectal cancer ($P_{trend} = .02$, Table 2). Among colorectal cancer patients, we observed higher mortality in patients with higher EDIP scores compared with those with lower scores ($P_{trend} = .002$, Supplementary Table 3). We conducted our primary hypothesis testing, and found that the association of EDIP scores with colorectal cancer risk significantly differed by the degrees of peritumoral lymphocytic reaction ($P_{heterogeneity} < .001$, with the adjusted α level of 0.01; Table 2). High EDIP scores were associated with higher risk of the colorectal cancer subtype with absent/low peritumoral lymphocytic reaction [highest vs lowest EDIP score quintile multivariable-adjusted HR = 2.60; 95% confidence interval (CI), 1.60–4.23; $P_{trend} < .001$], but not with risk of tumors that showed

intermediate or high peritumoral lymphocytic reaction (P_{trend} > .80). The association of EDIP score with colorectal cancer risk did not significantly differ by the degrees of Crohn's-like reaction, intratumoral periglandular reaction, or TIL ($P_{heterogeneity}$ > .03, with the adjusted α level of 0.01). Nonetheless, there was a positive association between EDIP scores and risk of the colorectal cancer subtype with absent/low intratumoral periglandular reaction (highest vs lowest EDIP score quintile multivariable-adjusted HR = 1.87; 95% CI, 1.15–3.05; P_{trend} = .003).

When the NHS and HPFS cohorts were analyzed separately, we found a stronger association between high EDIP scores and peritumoral lymphocytic reaction-absent/low colorectal cancer. Though statistical power was limited in these subgroup analyses, the differential association of EDIP scores with colorectal cancer subtypes classified by peritumoral reaction appeared to have similar trends in the two cohorts (Supplementary Table 4).

Because of the association of tumor molecular features such as MSI and CIMP status with lymphocytic reaction,[16] as well as a plausible influence of anti-inflammatory drugs on *BRAF*-wild-type or PTGS2-expressing colorectal cancers,[20, 27] we conducted secondary analyses that subclassified colorectal cancer subtypes (by peritumoral reaction) further by tumor MSI, CIMP, *BRAF*, or PTGS2 status (Table 3). Although statistical power was limited, the differential association of EDIP score with colorectal cancer subtypes classified by peritumoral lymphocytic reaction appeared to be generally consistent in non-MSI-high cases, CIMP-low/negative cases, *BRAF*-wild-type cases, PTGS2-negative cases, and PTGS2-positive cases. There were small numbers of cases in the other strata. Given the important role of MSI status in colorectal cancer in both research and clinical practice, we examined the associations of EDIP scores with risk of colorectal cancer subtypes by MSI status, and did not observe significant differential associations by MSI status (Supplementary Table 5).

Sensitivity Analyses

In sensitivity analyses that further adjusted for body mass index, we obtained similar results (Supplementary Table 6). Association measures between the EDIP score and risk of colorectal cancer subtypes classified by peritumoral lymphoctyic reaction remained significant in models that included each of the covariates in the multivariable-adjusted model. In addition, the association measures did not change materially even after adding each of interaction terms between the EDIP scores and individual potential confounders (alcohol intake and body mass index) into the multiple-adjusted model (Supplementary Table 7).

A sensitivity analysis was conducted to examine the relationship of the EDIP score (quartile categories) with risk of colorectal cancer subtypes classified by lymphocytic reaction, and similar findings were observed (Supplementary Table 8).

The findings from fitting marginal structural Cox proportional hazards models did not suggest significant time-varying confounding occurring after study enrollment. The fully weighted Cox proportional hazards models revealed a differential association between EDIP

scores and risk of colorectal cancer subtypes according to peritumoral lymphocytic reaction ($P_{heterogeneity}$ = .004; Supplementary Table 9).

DISCUSSION

Based on data from two large prospective cohorts of women and men, we found that high intakes of pro-inflammatory diets (indicated by high EDIP scores) were associated with a higher risk of developing colorectal cancer with absent/low peritumoral lymphocytic reaction, but not with risk of cancers that had intermediate or high peritumoral lymphocytic reaction. The positive associations between EDIP scores and risk of the colorectal cancer subtype with absent/low peritumoral lymphocytic reaction appeared to be consistent in non-MSI-high, CIMP-low/negative, *BRAF*-wild-type, PTGS2-negative, or PTGS2-positive colorectal cancer. The time-dependent Cox regression analyses revealed an immediate effect of cumulative average of EDIP scores on the development of colorectal cancer subtype with absent or low lymphocytic reaction. Although a validation in independent datasets is needed, our findings provide the first line of population-based evidence for the role of immunity in mediating the effect of dietary inflammatory potential in colorectal carcinogenesis. Hence, the current study may be of use in designing strategies for personalized immunoprevention through dietary interventions.

Analysis of exposures and tumor characteristics is increasingly important.[28–31] The positive association between dietary inflammatory potential and risk of the colorectal cancer subtype with absent/low peritumoral lymphocytic reaction is biologically plausible. Dietary components exert diverse influences on inflammation. Studies have shown that red meat intake can promote the secretion of IL6 and CRP.[32, 33] Saturated fatty acids can activate innate immune receptors, including Toll-like receptors and nucleotide-binding oligomerization domain proteins to upregulate pro-inflammatory cytokines IL1, IL6, and TNF (TNF-α), whereas, polyunsaturated fatty acids inhibit the secretion of these mediators.[34] Short chain fatty acids (SCFAs), byproducts of fermentation of dietary fiber, may reduce these pro-inflammatory cytokines and produce anti-inflammatory mediator IL10 through activation of free fatty acid receptors (FFAR2 and FFAR3) and inhibition of histone deacetylase of macrophages.[35, 36] The tryptophan metabolites derived from cruciferous vegetables, and carotenoids and flavonoids (which are abundant in yellow, orange, and red vegetables) increase the secretion of IL22 to maintain epithelial integrity through binding to the aryl hydrocarbon receptor expressed on intestinal dendritic cells and lymphocytes.[37] Studies have shown that pro-inflammatory dietary patterns, characterized by high saturated fatty acids, high sugar, high red and processed meat, low dietary fiber, and low green leafy and dark yellow vegetables, have been associated with high levels of circulating pro-inflammatory mediators.[6, 38, 39] In the acute or early phase of local intestinal inflammation, pro-inflammatory mediators mainly secreted by macrophages and mast cells recruit natural killer cells to eliminate pathogenic agents. Adaptive immune cells (T and B lymphocytes) are further activated by mature dendritic cells and undergo clonal expansion in order to mount an ‘adaptive’ response.[40] Systemic and local intestinal chronic inflammation caused by long-term intake of pro-inflammatory diets may cause activation of cellular signaling pathways related to phosphatidylinositol-4,5-bisphosphonate 3-kinase (PI3K), STAT3, and NFKB; all of these pathways are crucial in the early stage of tumorigenesis due to their

ability to stimulate proliferation and suppress apoptosis of pre-malignant cells.[5, 41, 42] Furthermore, chronic inflammation can dysregulate immune homeostasis and suppress adaptive anti-tumor immune response. The hyperactivation of PI3K / MTOR signaling may skew differentiation of $CD8^+$ T cells to short-lived effector cells with severely impaired development of B cells and memory T cells.[43] TGFB1 and STAT3 signaling can enhance regulatory T cell-mediated immunosuppression and result in impaired antigen-specific T-cell responses.[44] Persistent chronic inflammation can also result in deficient expression of CD28 in both $CD4^+$ and $CD8^+$ T cells.[45] $CD4^+$ $CD28^{null}$ T cells lose their capacity to help B cells due to the concomitant loss of CD40LG (CD154).[46] As a major CD40 ligand, CD40LG (CD154) is expressed by activated T lymphocytes. The interaction between CD40 and CD40LG could promote T cell dependent B cell proliferation, regulate B cell isotype switching and migration, and prolong the survival of antigen-specific high-affinity memory B cells.[47] A proportion of $CD8^+$ $CD28^{null}$ T cells lack perforin that is an important cytotoxic protein.[48] The number of $CD28^{null}$ T cells has been inversely correlated with protective immune responses.[49] With regard to peritumoral immune reaction, it has been shown that a strong in situ T lymphocytic reaction in invasive tumor margins correlated with a favorable prognosis independent of cancer stage.[50, 51] Consistent with these literature data, our findings suggest that inflammatory diets may promote the development of colorectal carcinomas with little or no peritumoral lymphocytic reaction. Whereas, colorectal cancer subtype with high lymphocytic reaction shows its immunogenic property due to tumor-producing neoantigens, and this immunogenic property of tumor may cause insensitivity to inflammatory diets, which have been shown to have suppressive effects on adaptive anti-tumor immunity. Hence, it is conceivable that the association between pro-inflammatory diets and risk of colorectal cancer differs among tumors subtypes with different degrees of immune responses to tumor. Our data have clinical implications, especially in terms of prevention of colorectal cancer. Reduced intake of inflammatory diets appears to be preventive against colorectal cancer subtype with absent/low lymphocytic reaction, which has been shown to be a clinically aggressive cancer subtype.[16] In addition, it can be hypothesized in the future studies that patients with colorectal cancer subtype exhibiting absent/low lymphocytic reaction may have benefits from intervention of anti-inflammatory diets after cancer diagnosis.

Integrated analysis of tumor molecular features and immune cells in the tumor microenvironment is increasingly important,[52–54] because ample evidence indicates bidirectional influences of tumor molecular alterations and immune response to tumor.[55, 56] Considering the relationship between tumor characteristics and immunity, we further investigated whether the observed association of pro-inflammatory diets with cancer containing little or no peritumoral lymphocytic reaction might be due to a potential association between pro-inflammatory diets and specific tumor molecular subtypes such as those classified by MSI, CIMP, *BRAF* mutation, or PTGS2 expression status. We found that the positive association between higher EDIP scores and risk of peritumoral lymphocytic reaction-absent/low colorectal cancer appeared to be consistent in non-MSI-high, CIMP-low, *BRAF*-wild, PTGS2-negative or PTGS2-positive colorectal cancers, while the numbers of cases were too small in strata of MSI-high, CIMP-high and *BRAF*-mutated tumors. Further

large-scale studies are needed to examine the relationship between diets and comprehensive tumor subtyping that integrates both tumor and immune characteristics.

There are several advantages to the approach taken in our study. First, our prospective diet data collection enabled us to not only minimize recall bias (that is inevitable in retrospective diet data collection) but also avoid differential recall bias between cancer cases and cancer-free participants. Second, repeated questionnaires enabled us to use cumulative averages for dietary intakes and all other quantitative factors and decrease measurement errors within individuals. Third, we applied the recently developed EDIP scores to assess dietary inflammatory potential. Hence, we analyzed overall dietary patterns that encompass many food items, rather than examining each food item individually which could exaggerate multiple comparisons and false discoveries. Fourth, the molecular pathological epidemiology (MPE) analysis method[23, 57] enabled us to assess differential association of inflammatory diets with incidence of cancer subtypes classified by immune features. This MPE method has been utilized to assess the combined influences of exposures and immunity in cancer occurrence.[11, 12, 58, 59]

Our current study has limitations. First, despite the large sample size from the two cohorts, the number of cases with absent/low peritumoral lymphocytic reaction was relatively small. Second, diet data were based on food frequency questionnaires. Despite the presence of measurement errors, food frequency questionnaires can capture long-term food intake habits better than diet diaries.[60] Third, we could not analyze all cases due to unavailability of tumor tissues in some cases. Considering that the tissue data may not be missing completely at random, we employed the inverse probability weighting method to adjust for potential bias related to tumor tissue data missingness. Fourth, our cohort participants were health professionals in the U.S., and mostly non-Hispanic Caucasians. Hence, generalizability of our findings in other population groups needs to be examined in future studies.

CONCLUSIONS

In summary, our current study has shown that pro-inflammatory diets are associated with a higher risk of colorectal cancer that contained little or no peritumoral lymphocytic reaction, but not risk of tumors with abundant peritumoral lymphocytic reaction. Although a validation by independent studies is needed, these findings suggest that pro-inflammatory diets may impair adaptive anti-tumor immune response, and hence promote colorectal carcinogenesis. Our data also implicate dietary inflammatory potential as an important factor to be considered in cancer immuno-prevention.[61, 62]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
CIMP	CpG island methylator phenotype
EDIP	empirical dietary inflammatory pattern
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
IPW	inverse probability weighting
MPE	molecular pathological epidemiology
MSI	microsatellite instability
NHS	Nurses' Health Study
TIL	tumor-infiltrating lymphocytes

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Table 1

Age-adjusted Baseline Characteristics of Participants across Quintiles of the Empirical Dietary Inflammatory Pattern Scores in the Pooled Cohorts of the Nurses' Health Study (Women, 1984) and the Health Professionals Follow-up Study (Men, 1986)[a]

Characteristic	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores				
	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)
Participants, No.	25,064	24,882	24,764	24,883	24,840
Age, years[b]	51.9 (7.9)	52.6 (8.3)	52.5 (8.5)	52.5 (8.7)	51.6 (8.7)
Race (white), %	96	96	95	94	93
Body mass index, kg/m^2	24.4 (3.5)	24.7 (3.7)	25.1 (4.0)	25.6 (4.4)	26.5 (5.1)
Family history of colorectal cancer, %	8	9	8	8	8
Smoking, pack-years	15.9 (19.3)	13.1 (17.9)	11.7 (17.3)	11.2 (17.4)	11.9 (18.2)
Waist hip ratio	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.7 (0.4)
Energy intake, kcal/day	1768 (539)	1696 (510)	1697 (507)	1765 (531)	1994 (592)
Total activity, METS-hours/week[c]	18.0 (25.5)	16.7 (23.4)	15.8 (22.7)	15.2 (22.4)	14.5 (22.0)
Current multivitamin use, %	41	40	39	38	36
History of endoscopy, %	35	35	34	35	34
Total alcohol intake, g/day	15.8 (17.4)	8.8 (11.5)	6.7 (10.2)	5.6 (9.5)	5.1 (10.0)
Regular aspirin use, %[d]	36	35	35	35	37
Food group components of the empirical dietary inflammatory pattern					
Processed meat, serving/day	0.25 (0.27)	0.26 (0.27)	0.29 (0.30)	0.34 (0.34)	0.51 (0.56)
Red meat, serving/day	0.53 (0.36)	0.55 (0.37)	0.58 (0.39)	0.65 (0.40)	0.82 (0.53)
Organ meat, serving/day	0.02 (0.04)	0.02 (0.04)	0.02 (0.04)	0.02 (0.04)	0.03 (0.05)
Other fish, serving/day	0.27 (0.22)	0.28 (0.22)	0.28 (0.23)	0.31 (0.25)	0.36 (0.34)
Other vegetable, serving/day	0.79 (0.64)	0.77 (0.58)	0.78 (0.60)	0.82 (0.64)	0.98 (0.92)
Refined grain, serving/day	0.92 (0.74)	1.00 (0.79)	1.12 (0.90)	1.33 (1.04)	1.88 (1.43)
High energy beverage, serving/day	0.15 (0.28)	0.19 (0.32)	0.24 (0.39)	0.32 (0.48)	0.69 (1.02)
Low energy beverage, serving/day	0.36 (0.65)	0.40 (0.68)	0.45 (0.74)	0.58 (0.90)	1.07 (1.65)
Tomato, serving/day	0.51 (0.40)	0.51 (0.39)	0.53 (0.39)	0.58 (0.42)	0.73 (0.68)
Beer, serving/day	0.38 (0.92)	0.17 (0.43)	0.11 (0.31)	0.08 (0.27)	0.07 (0.23)
Wine, serving/day	0.66 (0.96)	0.26 (0.37)	0.17 (0.27)	0.12 (0.21)	0.09 (0.19)

Characteristic	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores				
	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)
Tea, serving/day	0.62 (1.15)	0.62 (1.09)	0.60 (1.03)	0.58 (1.00)	0.55 (0.97)
Coffee, serving/day	3.80 (2.08)	2.76 (1.80)	2.04 (1.63)	1.58 (1.51)	1.25 (1.40)
Dark yellow vegetable, serving/day	0.37 (0.48)	0.32 (0.32)	0.30 (0.28)	0.29 (0.27)	0.28 (0.27)
Green leafy vegetable, serving/day	1.04 (0.92)	0.83 (0.59)	0.74 (0.53)	0.69 (0.50)	0.67 (0.53)
Snack, serving/day	0.80 (1.23)	0.61 (0.89)	0.54 (0.76)	0.52 (0.70)	0.56 (0.72)
Fruit juice, serving/day	0.85 (1.03)	0.79 (0.82)	0.74 (0.73)	0.71 (0.68)	0.69 (0.72)
Pizza, serving/day	0.10 (0.13)	0.07 (0.08)	0.07 (0.07)	0.06 (0.06)	0.06 (0.06)

Abbreviations: METS, metabolic equivalent task score.

[a]The mean ± standard deviation (SD) for continuous variables and percentage for categorical variables.

[b]All variables are age standardized except age.

[c]Physical activity is represented by the product sum of the METS of each specific recreational activity and hours spent on that activity per week.

[d]A standard tablet contains 325 mg aspirin, and regular users were defined as those who used at least two tablets per week.

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Table 2

Empirical Dietary Inflammatory Pattern Scores and Risk of Colorectal Cancer by Components of Lymphocytic Reaction in the Pooled Cohorts of the Nurses' Health Study (Women) and the Health Professionals Follow-up Study (Men)

Analysis	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					P_{trend}[a]	$P_{heterogeneity}$[b]
	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)		
Person-years	625,367	620,578	584,987	597,924	569,402		
Overall colorectal cancer							
N of cases (n=1311)	277	248	259	257	270		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.82 (0.72–0.93)	0.88 (0.77–0.99)	0.92 (0.81–1.04)	1.06 (0.94–1.20)	.17	
Multivariable HR (95% CI)[d]	1 (referent)	0.86 (0.75–0.98)	0.93 (0.82–1.06)	0.99 (0.87–1.13)	1.14 (0.99–1.30)	.02	
Crohn's-like lymphoid reaction							.55
Absent/low							
N of cases (n=813)	168	157	164	163	161		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.85 (0.68–1.07)	0.89 (0.71–1.11)	0.95 (0.77–1.19)	1.02 (0.82–1.28)	.65	
Multivariable HR (95% CI)[d]	1 (referent)	0.90 (0.72–1.12)	0.95 (0.76–1.19)	1.02 (0.82–1.28)	1.10 (0.88–1.38)	.28	
Intermediate							
N of cases (n=183)	37	39	28	33	46		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.90 (0.57–1.42)	0.67 (0.41–1.10)	0.87 (0.54–1.40)	1.39 (0.90–2.16)	.21	
Multivariable HR (95% CI)[d]	1 (referent)	0.93 (0.59–1.47)	0.71 (0.43–1.17)	0.92 (0.57–1.50)	1.47 (0.94–2.30)	.14	
High							
N of cases (n=80)	20	13	18	13	16		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.54 (0.27–1.10)	0.83 (0.44–1.57)	0.60 (0.29–1.24)	0.92 (0.47–1.79)	.74	
Multivariable HR (95% CI)[d]	1 (referent)	0.57 (0.28–1.14)	0.88 (0.47–1.63)	0.65 (0.31–1.34)	0.98 (0.51–1.91)	.87	
Peritumoral lymphocytic reaction							<.001
Absent/low							
N of cases (n=182)	27	31	32	41	51		
Age-adjusted HR (95% CI)[c]	1 (referent)	1.25 (0.73–2.14)	1.20 (0.70–2.05)	1.59 (0.96–2.63)	2.41 (1.49–3.90)	<.001	
Multivariable HR (95% CI)[d]	1 (referent)	1.31 (0.77–2.25)	1.28 (0.74–2.19)	1.71 (1.03–2.84)	2.60 (1.60–4.23)	<.001	

Analysis	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					P_{trend}[a]	$P_{heterogeneity}$[b]
	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)		
Intermediate							
N of cases (n=907)	205	171	176	171	184		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.73 (0.59–0.91)	0.78 (0.63–0.96)	0.81 (0.66–1.00)	0.92 (0.75–1.13)	.65	
Multivariable HR (95% CI)[d]	1 (referent)	0.77 (0.62–0.95)	0.84 (0.68–1.03)	0.88 (0.71–1.09)	0.99 (0.80–1.22)	.81	
High							
N of cases (n=216)	44	43	50	45	34		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.92 (0.60–1.40)	1.10 (0.73–1.67)	1.06 (0.70–1.62)	0.86 (0.54–1.37)	.79	
Multivariable HR (95% CI)[d]	1 (referent)	0.95 (0.63–1.44)	1.16 (0.77–1.75)	1.11 (0.73–1.70)	0.91 (0.57–1.45)	.99	
Intratumoral periglandular reaction							.04
Absent/low							
N of cases (n=164)	31	25	31	38	39		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.91 (0.53–1.58)	1.06 (0.63–1.80)	1.39 (0.84–2.28)	1.74 (1.07–2.84)	.007	
Multivariable HR (95% CI)[d]	1 (referent)	0.95 (0.55–1.65)	1.12 (0.66–1.90)	1.49 (0.91–2.46)	1.87 (1.15–3.05)	.003	
Intermediate							
N of cases (n=976)	216	185	188	182	205		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.78 (0.63–0.95)	0.80 (0.65–0.97)	0.82 (0.67–1.00)	0.98 (0.81–1.20)	.96	
Multivariable HR (95% CI)[d]	1 (referent)	0.81 (0.66–1.00)	0.85 (0.69–1.04)	0.88 (0.72–1.08)	1.06 (0.86–1.30)	.50	
High							
N of cases (n=170)	30	37	40	37	26		
Age-adjusted HR (95% CI)[c]	1 (referent)	1.01 (0.62–1.64)	1.26 (0.78–2.06)	1.23 (0.75–2.02)	0.90 (0.52–1.56)	.94	
Multivariable HR (95% CI)[d]	1 (referent)	1.04 (0.65–1.69)	1.34 (0.82–2.18)	1.30 (0.79–2.14)	0.96 (0.55–1.65)	.72	
Tumor-infiltrating lymphocytes							.52
Absent/low							
N of cases (n=984)	207	187	200	189	201		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.83 (0.67–1.02)	0.89 (0.73–1.09)	0.89 (0.73–1.09)	1.02 (0.84–1.25)	.69	
Multivariable HR (95% CI)[d]	1 (referent)	0.86 (0.70–1.06)	0.95 (0.77–1.16)	0.96 (0.78–1.18)	1.10 (0.90–1.35)	.27	
Intermediate							

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Analysis	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					P_{trend}[a]	$P_{heterogeneity}$[b]
	Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)		
N of cases (n=199)	41	38	34	40	46		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.90 (0.57–1.42)	0.82 (0.51–1.32)	1.07 (0.68–1.67)	1.33 (0.85–2.06)	.15	
Multivariable HR (95% CI)[d]	1 (referent)	0.94 (0.59–1.47)	0.87 (0.54–1.41)	1.14 (0.73–1.78)	1.41 (0.91–2.19)	.09	
High							
N of cases (n=128)	29	23	25	28	23		
Age-adjusted HR (95% CI)[c]	1 (referent)	0.65 (0.38–1.11)	0.83 (0.48–1.43)	0.95 (0.56–1.62)	0.95 (0.54–1.67)	.83	
Multivariable HR (95% CI)[d]	1 (referent)	0.68 (0.40–1.17)	0.88 (0.51–1.52)	1.02 (0.60–1.74)	1.03 (0.59–1.82)	.63	

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Linear trend test using the median value of each EDIP score quintile.

[b]The Wald test was used to test for the heterogeneity of the associations between the EDIP scores and colorectal cancer risk according to the components of lymphocytic reaction. The heterogeneity test was adjusted for time-varying pack-years of smoking (0 vs. 1–19 vs. 20–39 vs. ≥40 pack-years), family history of colorectal cancer, endoscopy status, physical activity level [quintiles of mean metabolic equivalent task score (METS) - hours per week], total energy intake (quintiles of kcal/day), total alcohol intake (0 vs. 1–5 vs. 6–15 vs. >15 g/day), current multivitamin use and regular aspirin use.

[c]Duplication-method Cox proportional cause-specific hazards regression weighted by inverse probabilities based on immune marker availability for competing risks data was used to compute HRs and 95% CIs. All analyses were stratified by age (in month), year of questionnaire return and sex.

[d]Multivariable HR was further adjusted for time-varying pack-years of smoking (0 vs. 1–19 vs. 20–39 vs. ≥40 pack-years), family history of colorectal cancer, endoscopy status, physical activity level (quintiles of METS - hours per week), total energy intake (quintiles of kcal/day), total alcohol intake (0 vs. 1–5 vs. 6–15 vs. >15 g/day), current multivitamin use and regular aspirin use.

Table 3

Empirical Dietary Inflammatory Pattern Scores and Risk of Colorectal Cancer by Microsatellite Instability, CpG Island Methylator Phenotype, *BRAF* Mutation, PTGS2 Expression and Peritumoral Lymphocytic Reaction

Tumor Characteristic	Peritumoral Lymphocytic Reaction	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					*P*trend[a]
		Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	
Microsatellite instability							
Non-MSI-high	Absent/low						
	N of cases (n=142)	23	26	23	32	38	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.18 (0.66–2.11)	0.96 (0.52–1.74)	1.47 (0.84–2.58)	2.19 (1.29–3.74)	.003
	Multivariable HR (95% CI)[c]	1 (referent)	1.24 (0.69–2.22)	1.03 (0.56–1.88)	1.60 (0.91–2.80)	2.40 (1.40–4.10)	<.001
	Intermediate						
	N of cases (n=705)	159	138	136	135	137	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.78 (0.61–0.98)	0.79 (0.62–1.00)	0.86 (0.68–1.09)	0.96 (0.76–1.22)	.99
	Multivariable HR (95% CI)[c]	1 (referent)	0.81 (0.64–1.03)	0.85 (0.67–1.08)	0.94 (0.74–1.19)	1.04 (0.82–1.32)	.48
	High						
	N of cases (n=113)	22	27	22	25	17	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.14 (0.65–2.00)	1.04 (0.57–1.89)	1.05 (0.58–1.89)	0.83 (0.42–1.62)	.57
	Multivariable HR (95% CI)[c]	1 (referent)	1.17 (0.67–2.05)	1.10 (0.61–2.00)	1.12 (0.62–2.01)	0.86 (0.45–1.67)	.70
MSI-high							
	Absent/low						
	N of cases (n=9)	0	1	2	5	1	
	Age-adjusted HR (95% CI)[b]	1 (referent)	-	-	-	-	-
	Multivariable HR (95% CI)[c]	1 (referent)	-	-	-	-	-
	Intermediate						
	N of cases (n=102)	21	16	25	18	22	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.68 (0.34–1.35)	1.09 (0.60–1.98)	0.77 (0.40–1.46)	1.05 (0.56–1.95)	.78
	Multivariable HR (95% CI)[c]	1 (referent)	0.72 (0.36–1.44)	1.17 (0.65–2.12)	0.83 (0.44–1.59)	1.13 (0.60–2.11)	.61
	High						
	N of cases (n=73)	16	15	19	11	12	

Tumor Characteristic	Peritumoral Lymphocytic Reaction	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					*P*trend[a]
		Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.83 (0.42–1.67)	1.08 (0.55–2.10)	0.74 (0.34–1.63)	0.89 (0.42–1.88)	.68
	Multivariable HR (95% CI)[c]	1 (referent)	0.88 (0.45–1.75)	1.15 (0.59–2.25)	0.79 (0.36–1.72)	0.97 (0.46–2.07)	.86
CpG island methylator phenotype (CIMP)							
CIMP-low/negative	Absent/low						
	N of cases (n=129)	19	23	23	31	33	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.33 (0.70–2.53)	1.26 (0.67–2.38)	1.70 (0.93–3.10)	2.38 (1.33–4.27)	.002
	Multivariable HR (95% CI)[c]	1 (referent)	1.41 (0.74–2.67)	1.36 (0.72–2.57)	1.84 (1.00–3.37)	2.57 (1.44–4.61)	<.001
	Intermediate						
	N of cases (n=681)	156	137	128	124	136	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.74 (0.59–0.94)	0.77 (0.60–0.98)	0.79 (0.62–1.01)	0.89 (0.70–1.13)	.48
	Multivariable HR (95% CI)[c]	1 (referent)	0.78 (0.61–0.99)	0.83 (0.65–1.06)	0.85 (0.66–1.09)	0.95 (0.75–1.21)	.89
	High						
	N of cases (n=105)	20	25	21	23	16	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.25 (0.69–2.26)	1.03 (0.54–1.97)	1.09 (0.59–2.01)	0.92 (0.46–1.86)	.80
	Multivariable HR (95% CI)[c]	1 (referent)	1.31 (0.73–2.36)	1.09 (0.57–2.08)	1.15 (0.62–2.13)	0.97 (0.48–1.92)	.92
CIMP-high							
	Absent/low						
	N of cases (n=16)	3	4	2	4	3	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.66 (0.34–8.18)	0.83 (0.14–4.92)	1.64 (0.36–7.52)	1.92 (0.38–9.60)	.44
	Multivariable HR (95% CI)[c]	1 (referent)	1.67 (0.34–8.26)	0.85 (0.14–5.05)	1.78 (0.38–8.24)	2.02 (0.40–10.16)	.39
	Intermediate						
	N of cases (n=110)	22	16	25	25	22	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.70 (0.36–1.38)	0.95 (0.53–1.72)	1.08 (0.60–1.93)	1.07 (0.58–1.98)	.59
	Multivariable HR (95% CI)[c]	1 (referent)	0.75 (0.38–1.47)	1.03 (0.58–1.85)	1.19 (0.67–2.13)	1.13 (0.61–2.08)	.44
	High						
	N of cases (n=64)	13	15	17	10	9	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.83 (0.40–1.72)	1.13 (0.55–2.33)	0.75 (0.32–1.74)	0.64 (0.27–1.51)	.26

Tumor Characteristic	Peritumoral Lymphocytic Reaction	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					*P*trend[a]
		Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	
	Multivariable HR (95% CI)[c]	1 (referent)	0.87 (0.43–1.79)	1.22 (0.60–2.49)	0.78 (0.33–1.81)	0.69 (0.29–1.62)	.34
BRAF							
Wild-type	Absent/low						
	N of cases (n=130)	20	22	21	30	37	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.19 (0.64–2.24)	1.13 (0.60–2.13)	1.56 (0.86–2.82)	2.61 (1.49–4.58)	<.001
	Multivariable HR (95% CI)[c]	1 (referent)	1.27 (0.68–2.38)	1.23 (0.65–2.31)	1.71 (0.94–3.10)	2.85 (1.63–4.99)	<.001
	Intermediate						
	N of cases (n=717)	155	141	143	135	143	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.79 (0.62–1.00)	0.87 (0.69–1.10)	0.87 (0.69–1.11)	1.00 (0.79–1.27)	.75
	Multivariable HR (95% CI)[c]	1 (referent)	0.84 (0.66–1.06)	0.94 (0.74–1.19)	0.95 (0.75–1.22)	1.08 (0.85–1.37)	.33
	High						
	N of cases (n=139)	27	31	32	29	20	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.05 (0.63–1.77)	1.14 (0.67–1.94)	0.97 (0.57–1.66)	0.79 (0.42–1.47)	.43
	Multivariable HR (95% CI)[c]	1 (referent)	1.11 (0.67–1.87)	1.22 (0.72–2.07)	1.05 (0.61–1.79)	0.83 (0.45–1.53)	.58
Mutant							
	Absent/low						
	N of cases (n=20)	3	5	4	5	3	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.42 (0.33–6.08)	0.77 (0.17–3.38)	1.25 (0.31–5.05)	0.87 (0.18–4.10)	.87
	Multivariable HR (95% CI)[c]	1 (referent)	1.47 (0.35–6.20)	0.82 (0.19–3.65)	1.34 (0.33–5.50)	0.95 (0.20–4.54)	.99
	Intermediate						
	N of cases (n=101)	29	16	18	18	20	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.50 (0.27–0.93)	0.50 (0.27–0.91)	0.57 (0.31–1.05)	0.85 (0.48–1.52)	.60
	Multivariable HR (95% CI)[c]	1 (referent)	0.53 (0.28–0.98)	0.53 (0.29–0.97)	0.62 (0.34–1.14)	0.93 (0.52–1.65)	.78
	High						
	N of cases (n=48)	11	12	11	7	7	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.87 (0.39–1.94)	0.86 (0.38–1.98)	0.73 (0.27–1.92)	0.82 (0.32–2.11)	.56
	Multivariable HR (95% CI)[c]	1 (referent)	0.89 (0.40–1.97)	0.92 (0.40–2.10)	0.77 (0.29–2.02)	0.89 (0.34–2.29)	.69

Tumor Characteristic	Peritumoral Lymphocytic Reaction	Quintiles of the Empirical Dietary Inflammatory Pattern (EDIP) Scores					*P*trend[a]
		Q1 (Lowest)	Q2	Q3	Q4	Q5 (Highest)	
PTGS2 expression							
Negative							
	Absent/low	8	16	11	8	13	
	N of cases (n=56)	1 (referent)	2.42 (0.96–6.10)	1.68 (0.63–4.43)	1.45 (0.52–4.04)	2.25 (0.87–5.81)	.32
	Age-adjusted HR (95% CI)[b]	1 (referent)	2.62 (1.05–6.54)	1.84 (0.70–4.87)	1.63 (0.59–4.50)	2.49 (0.97–6.40)	.20
	Multivariable HR (95% CI)[c]						
	Intermediate						
	N of cases (n=281)	65	44	66	51	55	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.59 (0.39–0.88)	0.85 (0.59–1.23)	0.71 (0.48–1.03)	0.87 (0.60–1.28)	.71
	Multivariable HR (95% CI)[c]	1 (referent)	0.62 (0.41–0.93)	0.92 (0.64–1.33)	0.76 (0.52–1.11)	0.95 (0.65–1.39)	.97
	High						
	N of cases (n=73)	15	20	17	11	10	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.01 (0.52–1.95)	1.05 (0.51–2.17)	0.66 (0.29–1.51)	0.75 (0.33–1.71)	.29
	Multivariable HR (95% CI)[c]	1 (referent)	1.07 (0.56–2.04)	1.14 (0.55–2.33)	0.71 (0.31–1.61)	0.80 (0.35–1.82)	.38
Positive							
	Absent/low						
	N of cases (n=80)	14	7	13	22	24	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.49 (0.19–1.29)	0.88 (0.39–1.97)	1.49 (0.73–3.08)	2.28 (1.16–4.50)	.003
	Multivariable HR (95% CI)[c]	1 (referent)	0.52 (0.20–1.35)	0.95 (0.42–2.11)	1.62 (0.78–3.35)	2.46 (1.24–4.85)	.001
	Intermediate						
	N of cases (n=501)	105	105	94	91	106	
	Age-adjusted HR (95% CI)[b]	1 (referent)	0.85 (0.64–1.12)	0.88 (0.66–1.18)	0.85 (0.63–1.14)	1.01 (0.76–1.34)	.97
	Multivariable HR (95% CI)[c]	1 (referent)	0.90 (0.67–1.19)	0.95 (0.71–1.28)	0.93 (0.69–1.25)	1.08 (0.81–1.44)	.65
	High						
	N of cases (n=82)	12	16	22	16	16	
	Age-adjusted HR (95% CI)[b]	1 (referent)	1.29 (0.59–2.82)	1.58 (0.75–3.31)	1.08 (0.50–2.37)	1.09 (0.50–2.37)	.79
	Multivariable HR (95% CI)[c]	1 (referent)	1.34 (0.61–2.92)	1.67 (0.80–3.48)	1.15 (0.52–2.51)	1.14 (0.53–2.48)	.67

Abbreviations: CI, confidence interval; CIMP, CpG island methylator phenotype; HR, hazard ratio; MSI, microsatellite instability.

[a] Linear trend test using the median value of each EDIP score quintile.

[b] Duplication-method Cox proportional cause-specific hazards regression weighted by inverse probabilities based on immune marker availability for competing risks data was used to compute HRs and 95% CIs. All analyses were stratified by age (in month), year of questionnaire return and sex.

[c] Multivariable HR was further adjusted for time-varying pack-years of smoking (0 vs. 1–19 vs. 20–39 vs. ≥40 pack-years), family history of colorectal cancer, endoscopy status, physical activity level [quintiles of mean metabolic equivalent task score (METS) - hours per week], total energy intake (quintiles of kcal/day), total alcohol intake (0 vs. 1–5 vs. 6–15 vs. >15 g/day), current multivitamin use and regular aspirin use.