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MicroRNA *MIR21* (miR-21) and PTGS2 Expression in Colorectal Cancer and Patient Survival

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Abstract

Purpose: Prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase-2; a target of aspirin) produces inflammatory mediator prostaglandin E_2 (PGE_2), and contributes to colorectal neoplasia development. PTGS2-driven inflammatory responses can induce tumor expression of microRNA *MIR21* (miR-21) that can increase local PGE_2 level by downregulating PGE_2-metabolizing enzymes. We hypothesized that the prognostic association of tumor *MIR21* expression level in colorectal carcinoma might depend on inflammatory tumor microenvironment and be stronger in tumors expressing high-level PTGS2.

Experimental Design: Utilizing 765 rectal and colon cancer specimens in the Nurses' Health Study and the Health Professionals Follow-up Study, we measured *MIR21* expression by quantitative reverse transcription PCR, and PTGS2 expression by immunohistochemistry. Cox proportional hazards regression model was used to assess statistical interaction between *MIR21* and PTGS2 in colorectal cancer–specific survival analysis, controlling for potential confounders including microsatellite instability, CpG island methylator phenotype, LINE-1 methylation level, and *KRAS*, *BRAF*, and *PIK3CA* mutations.

Results: Tumor *MIR21* expression level was associated with higher colorectal cancer–specific mortality ($P_{trend} = 0.029$), and there was a statistically significant interaction between *MIR21* and PTGS2 ($P_{interaction} = 0.0004$). The association between *MIR21* expression and colorectal cancer–specific mortality was statistically significant in PTGS2-high cancers (multivariable hazard ratio of the highest vs. lowest quartile of *MIR21*, 2.28; 95% confidence interval, 1.42–3.67; $P_{trend} = 0.0004$) but not in PTGS2-absent/low cancers ($P_{trend} = 0.22$).

Conclusions: *MIR21* expression level in colorectal carcinoma is associated with worse clinical outcome, and this association is stronger in carcinomas expressing high-level PTGS2, suggesting complex roles of immunity and inflammation in tumor progression. *Clin Cancer Res; 1–8. ©2016 AACR.*

Introduction

Colorectal cancers develop through the accumulation of genetic and epigenetic alterations and through tumor–host interactions including inflammatory responses and host immunity (1, 2). Prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase-2) produces inflammatory mediator prostaglandin E_2 (PGE_2) and contributes to colorectal tumor development and progression (3–5). Randomized controlled trials and observational studies have demonstrated that regular use of aspirin (PTGS2 inhibitor) reduces the risk of colorectal neoplasia incidence and mortality (6, 7). Levels of PGE_2 in the tumor microenvironment are likely influenced by PTGS2 that produces PGE_2 (3). Previous studies suggest that cellular PTGS2 expression may influence effects of aspirin and selective inhibitors of PTGS2 on colorectal tumors (8, 9).

MicroRNAs (miRNAs) are small noncoding RNAs that post-transcriptionally regulate gene expression and have been shown to influence diverse physiologic and pathologic processes, including immunity, inflammation, and carcinogenesis (10). Accumulating evidence indicates that inflammatory responses can alter expression of miRNAs, some of which may contribute to tumor progression (11, 12). Among those miRNAs, *MIR21* (miR-21) has been shown to promote inflammation-associated colorectal

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Translational Relevance

Accumulating evidence indicates that microRNAs are promising biomarkers and therapeutic targets in cancer. We examined an association of tumor *MIR21* expression level with patient survival utilizing 765 colorectal cancer cases in two U.S. nationwide prospective cohort studies (the Nurses' Health Study and the Health Professionals Follow-up Study). We found that tumor *MIR21* expression level was associated with higher colorectal cancer–specific mortality independent of clinical, pathologic, and major tumor molecular features, including microsatellite instability, CpG island methylator phenotype, *KRAS*, *BRAF*, and *PIK3CA* mutations, and LINE-1 methylation level. In addition, this adverse prognostic association was stronger in colorectal cancers expressing high-level prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase-2) that produces inflammatory mediator prostaglandin E_2. Our population-based data suggest that *MIR21* may serve as a potential therapeutic target, especially for colorectal cancers that express PTGS2 and may depend on inflammatory tumor microenvironment.

tumorigenesis in animal models (13, 14). In addition, studies of human colorectal cancer tissue have shown that *MIR21* is upregulated in colorectal cancer cells, and that tumor *MIR21* expression level is associated with high-level expression of the genes involved in inflammatory responses and worse clinical outcome (15, 16). *MIR21* appears to downregulate gene products that catalyze degradation of PGE_2, which leads to increased level of PGE_2 in the tumor microenvironment and promotes tumor growth in a xenograft model (17). Hence, there might be a synergistic effect of *MIR21* and PTGS2 on tumor progression. We hypothesized that the association of tumor *MIR21* expression level with worse clinical outcome in colorectal cancer might be stronger in cancers expressing high-level PTGS2.

To test this hypothesis, we utilized resources of 765 colorectal cancer cases in two U.S. nationwide prospective cohort studies [the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS)], and examined a statistical interaction between tumor *MIR21* and PTGS2 expression in survival analysis, controlling for potential confounders including major molecular features of colorectal cancer.

Materials and Methods

Study population

We utilized the database of colorectal carcinoma cases within two U.S. nationwide prospective cohort studies, the NHS (121,701 women who enrolled in 1976) and the HPFS (51,529 men who enrolled in 1986; refs.18, 19). Every 2 years, participants were sent follow-up questionnaires to collect information on health and lifestyle factors, and asked whether they had received diagnoses of major diseases including cancer. The National Death Index was used to ascertain deaths of study participants and identify unreported lethal colorectal cancer cases. For incident colorectal cancer cases, medical records were reviewed. If a patient was deceased, the cause of death was assigned by study physicians. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected from hospitals where participants with colorectal cancer had undergone tumor resection. A single pathologist (S. Ogino), who was unaware of other data, conducted a centralized review of hematoxylin and eosin–stained tissue sections of all colorectal carcinoma cases, and recorded pathologic features. Tumor differentiation was categorized as well to moderate or poor (>50% vs. ≤50% glandular area). We analyzed available data on tumor *MIR21* and PTGS2 expression and patient survival in 765 patients diagnosed up to 2008. Patients were followed until death or January 1, 2012, whichever came first. Written informed consent was obtained from all study participants. The procedures and protocols of this study were approved by the institutional review boards for the Harvard T.H. Chan School of Public Health and the Brigham and Women's Hospital (Boston, MA).

RNA isolation and quantitative reverse transcription PCR for *MIR21*

RNA was extracted from colorectal cancer tissue in whole-tissue sections of FFPE specimens with the use of RecoverAll Total Nucleic Acid Isolation Kit (Ambion Inc). Quantitative reverse transcription PCR assays for *MIR21* and *RNU6-2* were performed according to miScript PCR System protocol (Qiagen) after assay validation as described previously (20). Briefly, cDNA was synthesized with the use of miScript II RT Kit (Qiagen). Each reaction was performed in 25 μL solution containing 1× final concentration QuantiTect SYBR Green PCR Master Mix (Qiagen) and each miScript Primer Assay (Qiagen) specific for *MIR21* (catalog number, MS00009079) and *RNU6-2* (catalog number, MS00033740) in a 96-well optical PCR plate. Amplification and detection of *MIR21* and *RNU6-2* were performed with the StepOnePlus Real-Time PCR Systems (Applied Biosystems) with the use of the following reaction conditions: 15 minutes at 95°C and 40 cycles of 15 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 70°C.

Our validation study has previously shown that the cycle threshold (C_t) values in the quantitative reverse transcription PCR assays for *MIR21* and *RNU6-2* decreased linearly with the amount of input cDNA using 10-fold dilution series from the same specimen ($r^2 > 0.99$), and that the inter-assay coefficient of variation of C_t values from the same specimen in five different batches was ≤1% for *MIR21* and *RNU6-2* (20). Each specimen was analyzed in duplicate for each target in a single batch, and we used the mean of the two C_t values for each target. Spearman's rank-correlation coefficient between the two C_t values (in duplicated runs) was 0.99 in quantitative PCR assays for *MIR21* and *RNU6-2* (20). *MIR21* expression level in each specimen was calculated as a relative unitless value normalized with *RNU6-2* using the $2^{-\Delta C_t}$ method (where ΔC_t = "the mean C_t value of *MIR21*" − "the mean C_t value of *RNU6-2*") as described previously (20).

Immunohistochemistry for PTGS2 expression

Immunohistochemistry (IHC) for PTGS2 (cyclooxygenase-2) was performed using anti-PTGS2 antibody (Cayman Chemical; dilution 1:300) as described previously (5, 8). A single pathologist (S. Ogino), unaware of other data, interpreted tumor PTGS2 expression level (absent, low, or high), compared with adjacent normal colonic epithelium. A random sample of 124 cancers was examined by a second pathologist (T. Morikawa), and concordance between the two observers was 0.85 ($\kappa = 0.69$; ref. 18).

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Representative sections from PTGS2-absent, PTGS2-low, and PTGS2-high tumors have been shown in our previous study (5).

Analyses of MSI, DNA methylation, and *KRAS*, *BRAF*, and *PIK3CA* mutations

DNA was extracted from archival colorectal cancer tissue blocks. Microsatellite instability (MSI) status was analyzed with use of 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) as described previously (21). We defined MSI-high as the presence of instability in ≥30% of the markers, and MSI-low/microsatellite stable (MSS) as instability in <30% of the markers. Methylation analyses of long interspersed nucleotide element-1 (LINE-1; refs. 22, 23) and eight promoter CpG islands specific for CpG island methylator phenotype (CIMP; *CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*; refs. 24, 25) were performed. PCR reaction and pyrosequencing were performed for *KRAS* (codons 12, 13, 61, and 146; refs. 26, 27), *BRAF* (codon 600; ref. 21), and *PIK3CA* (exons 9 and 20; ref. 28).

Statistical analysis

All statistical analyses were conducted using SAS (version 9.3, SAS Institute) and all *P* values were two-sided. Our primary hypothesis testing was a statistical interaction between tumor *MIR21* and PTGS2 expression in relation to colorectal cancer–specific mortality. Neither *MIR21* expression nor log-transformed values of *MIR21* fit a normal distribution with the use of the Kolmogorov–Smirnov test for normality ($P < 0.01$). We conducted statistical test for a linear trend across ordinal quartile categories (1–4) of the tumor *MIR21* expression level as a continuous variable in the Cox regression model. All other analyses including evaluation of individual hazard ratio (HR) estimates were secondary analyses. The statistical interaction was assessed by the Wald test on the cross-product term of tumor *MIR21* expression [ordinal quartile categories (1–4)] and PTGS2 expression [ordinal categories; absent (1), low (2), and high (3)] variables in a Cox proportional hazards regression model. A two-sided α level was set at 0.05 for our primary hypothesis testing. For all of the primary and secondary analyses, we interpreted our results cautiously, given the exploratory hypothesis-generating nature of this study.

For analyses of colorectal cancer–specific mortality, deaths as a result of other causes were censored. To control for confounding, we used multivariable Cox proportional hazards regression models. In addition to tumor *MIR21* expression level, the multivariable model initially included sex, age at diagnosis (continuous), year of diagnosis (continuous), family history of colorectal cancer in a first-degree relative (present vs. absent), tumor location (proximal colon vs. distal colon vs. rectum), disease stage (I/II vs. III/IV), tumor differentiation (well/moderate vs. poor), MSI (high vs. MSI-low/MSS), CIMP (high vs. low/negative), *KRAS* (mutant vs. wild-type), *BRAF* (mutant vs. wild-type), *PIK3CA* (mutant vs. wild-type), and tumor LINE-1 methylation level (continuous). A backward elimination was carried out with $P = 0.05$ as a threshold, to select variables for the final model. For cases (3.0%) with missing information on LINE-1 methylation level, we assigned a separate indicator variable. For cases with missing information in any of the categorical covariates [family history of colorectal cancer in a first-degree relative (0.4%), tumor location (0.4%), disease stage (5.9%), tumor differentiation (0.1%), MSI (3.4%), CIMP (7.2%), *KRAS* (3.0%), *BRAF* (2.4%), and *PIK3CA* (9.4%)], we included these cases in the majority category of a given covariate to minimize the number of variables in multivariable Cox models. We confirmed that excluding the cases with missing information in any of the covariates did not substantially alter results (data not shown). The proportionality of hazards assumption was assessed by a time-varying covariate, using an interaction term of survival time and tumor *MIR21* expression level variable ($P = 0.63$ for colorectal cancer–specific mortality and $P = 0.11$ for overall mortality). The Kaplan–Meier method was used to describe the distribution of colorectal cancer–specific survival and overall survival, and the log-rank test for trend was performed to assess a linear trend in survival probability across the ordinal quartile categories of tumor *MIR21* expression level.

All cross-sectional univariable analyses for clinical, pathologic, and tumor molecular associations were secondary analyses, and we adjusted two-sided α level to 0.003 (= 0.05/15) by simple Bonferroni correction for multiple hypothesis testing. To assess associations between categorical data, the χ^2 test was performed. To compare mean age and mean LINE-1 methylation levels, an ANOVA assuming equal variances was performed.

Results

Clinical, pathologic, and tumor molecular associations

To test our primary hypothesis on the statistical interaction between tumor *MIR21* and PTGS2 expression in colorectal cancer–specific survival analysis, we utilized the database of 765 colorectal cancer cases within the two prospective cohort studies. We measured tumor *MIR21* expression level, using the quantitative reverse transcription PCR assay as described previously (20). Table 1 summarizes clinical, pathologic, and tumor molecular features according to tumor *MIR21* expression level. High-level tumor *MIR21* expression was associated with higher disease stage and *BRAF* mutation ($P \leq 0.0008$ with adjusted α level of 0.003 for multiple hypothesis testing).

Association of tumor *MIR21* expression level with colorectal cancer mortality

We examined the relationship between tumor *MIR21* expression level and colorectal cancer mortality. In the 765 colorectal cancer cases, there were 429 deaths, including 231 colorectal cancer–specific deaths, during a median follow-up of 12.6 years (interquartile range: 9.8–17.3 years) for censored cases. In Kaplan–Meier analysis, tumor *MIR21* expression level was associated with higher colorectal cancer–specific mortality ($P = 0.0008$ by the log-rank test for trend) and overall mortality ($P = 0.001$ by the log-rank test for trend; Fig. 1). Tumor *MIR21* expression level was associated with higher colorectal cancer–specific mortality in univariable ($P_{trend} = 0.0008$) and multivariable Cox regression analyses ($P_{trend} = 0.029$; Table 2).

Interactive association of tumor *MIR21* and PTGS2 expression level in survival analysis

In our primary hypothesis testing, we found a statistically significant interaction between tumor *MIR21* and PTGS2 expression level in colorectal cancer–specific survival analysis ($P_{interaction} = 0.0004$; Table 3). Tumor *MIR21* expression level was significantly associated with higher colorectal cancer–specific mortality in PTGS2-high cancers ($P_{trend} = 0.0004$) but not in PTGS2-absent/low cancers ($P_{trend} = 0.22$). Multivariable HRs

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Table 1. Clinical, pathologic, and tumor molecular features according to tumor *MIR21* expression level in 765 colorectal cancer cases

		Tumor *MIR21* expression level (quartile)				
Characteristics[a]	**Total no. (*n* = 765)**	**Quartile 1 (lowest) (*n* = 192)**	**Quartile 2 (*n* = 190)**	**Quartile 3 (*n* = 192)**	**Quartile 4 (highest) (*n* = 191)**	***P*[b]**
Mean age ± SD (year)	68.5 ± 8.7	67.2 ± 8.6	68.7 ± 9.1	69.5 ± 7.8	68.5 ± 9.3	0.07
Sex						0.052
Men	328 (43%)	84 (44%)	79 (42%)	96 (50%)	69 (36%)	
Women	437 (57%)	108 (56%)	111 (58%)	96 (50%)	122 (64%)	
Year of diagnosis						0.014
Prior to 1995	276 (36%)	88 (46%)	70 (37%)	58 (30%)	60 (31%)	
1996–2000	250 (33%)	54 (28%)	66 (35%)	60 (31%)	70 (37%)	
2001–2008	239 (31%)	50 (26%)	54 (28%)	74 (39%)	61 (32%)	
Family history of colorectal cancer in a first-degree relative						0.40
Absent	605 (79%)	148 (77%)	158 (84%)	151 (79%)	148 (78%)	
Present	157 (21%)	44 (23%)	31 (16%)	40 (21%)	42 (22%)	
Tumor location						0.013
Cecum	134 (18%)	25 (13%)	30 (16%)	41 (21%)	38 (20%)	
Ascending to transverse colon	242 (32%)	48 (25%)	59 (31%)	62 (32%)	73 (39%)	
Splenic flexure to sigmoid	216 (28%)	66 (35%)	60 (32%)	44 (23%)	46 (24%)	
Rectosigmoid and rectum	170 (22%)	52 (27%)	40 (21%)	45 (24%)	33 (17%)	
Disease stage						0.0008
I	171 (24%)	56 (32%)	45 (25%)	39 (21%)	31 (17%)	
II	236 (33%)	56 (32%)	65 (37%)	60 (33%)	55 (30%)	
III	213 (29%)	39 (22%)	50 (28%)	66 (36%)	58 (32%)	
IV	100 (14%)	26 (14%)	18 (10%)	17 (9.3%)	39 (21%)	
Tumor differentiation						0.15
Well/moderate	694 (91%)	173 (91%)	179 (94%)	175 (91%)	167 (87%)	
Poor	70 (9.2%)	18 (9.4%)	11 (5.8%)	17 (8.9%)	24 (13%)	
PTGS2 expression						0.92
Absent	125 (16%)	33 (17%)	26 (14%)	34 (18%)	32 (17%)	
Low	170 (22%)	40 (21%)	47 (25%)	42 (22%)	41 (21%)	
High	470 (62%)	119 (62%)	117 (61%)	116 (60%)	118 (62%)	
MSI status						0.032
MSI-low/MSS	627 (85%)	169 (91%)	156 (85%)	154 (84%)	148 (80%)	
MSI-high	112 (15%)	17 (9.1%)	28 (15%)	30 (16%)	37 (20%)	
MLH1 hypermethylation						0.19
Absent	617 (87%)	162 (91%)	158 (88%)	147 (84%)	150 (85%)	
Present	93 (13%)	16 (9.0%)	22 (12%)	28 (16%)	27 (15%)	
CIMP status						0.020
Low/negative	582 (82%)	154 (87%)	154 (86%)	141 (81%)	133 (75%)	
High	128 (18%)	24 (13%)	26 (14%)	34 (19%)	44 (25%)	
BRAF mutation						0.0007
Wild-type	629 (84%)	171 (91%)	163 (87%)	154 (82%)	141 (76%)	
Mutant	118 (16%)	17 (9.0%)	24 (13%)	33 (18%)	44 (24%)	
KRAS mutation						0.019
Wild-type	448 (60%)	112 (60%)	97 (52%)	128 (68%)	111 (60%)	
Mutant	294 (40%)	74 (40%)	88 (48%)	59 (32%)	73 (40%)	
PIK3CA mutation						0.78
Wild-type	577 (83%)	142 (85%)	149 (83%)	144 (81%)	142 (85%)	
Mutant	116 (17%)	26 (15%)	30 (17%)	34 (19%)	26 (15%)	
Mean LINE-1 methylation level (%) ± SD	62.1 ± 9.3	61.0 ± 9.0	60.7 ± 9.8	62.7 ± 9.3	63.8 ± 8.9	0.004

Abbreviations: CIMP, CpG island methylator phenotype; LINE-1, long interspersed nucleotide element-1.

[a]Percentage (%) indicates the proportion of cases with a specific clinical, pathologic, or tumor molecular feature in colorectal cancer cases with each quartile category of tumor *MIR21* expression level. There were cases that had missing values for any of the characteristics except for age, sex, and year of diagnosis.

[b]To assess associations between the ordinal quartile categories of tumor *MIR21* expression level and categorical data, the χ^2 test was performed. To compare mean age and mean LINE-1 methylation levels, an ANOVA was performed. We adjusted two-sided α level to 0.003 (= 0.05/15) by simple Bonferroni correction for multiple hypothesis testing.

of the highest versus lowest quartile of *MIR21* expression for colorectal cancer–specific mortality were 2.28 [95% confidence interval (CI), 1.42–3.67) in PTGS2-high cancers and 0.61 (95% CI, 0.34–1.10) in PTGS2-absent/low cancers (Table 3).

Interaction of tumor *MIR21* expression level and regular aspirin use after diagnosis in survival analysis of stage I to III patients

As a secondary analysis, we examined the relationship between regular aspirin use after diagnosis and colorectal cancer mortality according to tumor *MIR21* expression level among 579 patients with stage I to III colorectal cancer (Supplementary Methods and Supplementary Table S1). No statistically significant interaction between tumor *MIR21* expression level and postdiagnosis aspirin use was observed in colorectal cancer–specific or overall survival analysis ($P_{interaction} > 0.20$; Supplementary Table S1); however, statistical power was limited.

Discussion

We conducted this study to test the hypothesis that the association of tumor *MIR21* expression level in colorectal cancer tissue

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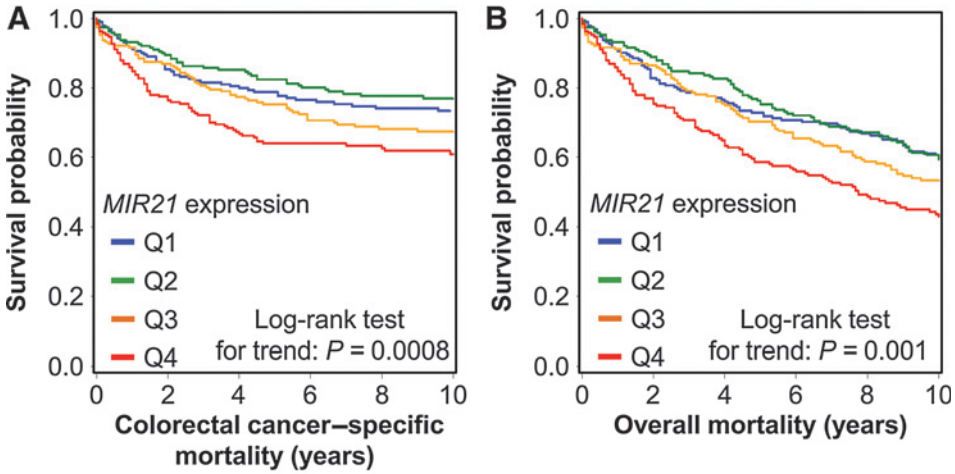

Figure 1.
Kaplan–Meier curves for colorectal cancer–specific mortality (A) and overall mortality (B) according to tumor *MIR21* expression level. *P* values were calculated by the log-rank test for trend (two-sided). The tables (bottom) show the number of patients who remained alive and at risk of death at each time point after the diagnosis of colorectal cancer. Q1 to Q4, quartile 1 to quartile 4.

Number at risk

MIR21 expression	Year					
	0	2	4	6	8	10
Q1	192	159	146	131	120	96
Q2	190	169	157	134	119	93
Q3	192	166	144	121	102	82
Q4	191	144	121	104	85	61

with worse clinical outcome might be stronger in cancers expressing high-level PTGS2. Utilizing the database of the 765 colorectal cancer cases in the two U.S. nationwide prospective cohort studies, we found that tumor *MIR21* expression level was associated with higher colorectal cancer–specific mortality, consistent with previous studies by other investigators (15). Our population-based data have provided evidence for the prognostic significance of tumor *MIR21* expression level in colorectal cancer, independent of clinical, pathologic, and major tumor molecular features. In addition, there was a statistically significant interaction between tumor *MIR21* and PTGS2 expression level in the survival analysis. As we hypothesized, the adverse prognostic association of tumor *MIR21* expression level in colorectal cancer was stronger in PTGS2-high cancers than in PTGS2-absent/low cancers. In our secondary analysis, there was no significant difference in the prognostic association of postdiagnosis aspirin use by *MIR21* expression level. However, statistical power was limited in our analysis of stage I to III patients, to minimize ascertainment bias in aspirin use data collection after cancer diagnosis.

Colorectal cancers are a heterogeneous group of diseases that result from the accumulation of differing sets of genomic and epigenomic alterations, and tumor–host interactions (29–35). Therefore, research on tumor biomarkers is important for clinical medicine and public health (36–39). In the current study, high-level tumor *MIR21* expression was associated with *BRAF* mutation, which has been associated with clinical outcome in colorectal cancer (40–43). An integrative analysis of multiple gene expression datasets of colorectal cancer by Guinney and colleagues (44) has suggested four major tumor subtypes. The majority of *BRAF*-mutated colorectal cancers have been included in one tumor subtype that is also associated with MSI-high and high-level antitumor immunity. Our current study has shown the association of *BRAF* mutation in colorectal cancer with high-level tumor *MIR21* expression, which may potentiate the PTGS2/PGE_2 pathway and suppress antitumor immunity (20). However, lack of gene expression profiling data precluded our use of colorectal cancer subtyping scheme described by Guinney and colleagues (44).

Table 2. Tumor *MIR21* expression level and colorectal cancer mortality

		Colorectal cancer–specific mortality			Overall mortality		
	No. of cases	No. of events	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]	No. of events	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]
MIR21 expression level							
Quartile 1 (lowest)	192	51	1 (reference)	1 (reference)	104	1 (reference)	1 (reference)
Quartile 2	190	44	0.85 (0.57–1.27)	0.88 (0.58–1.31)	98	0.99 (0.75–1.31)	0.99 (0.75–1.31)
Quartile 3	192	61	1.24 (0.85–1.80)	1.10 (0.75–1.60)	106	1.17 (0.89–1.53)	1.03 (0.78–1.35)
Quartile 4 (highest)	191	75	1.67 (1.17–2.39)	1.42 (0.98–2.04)	121	1.52 (1.16–1.97)	1.40 (1.07–1.84)
P_{trend}[b]			0.0008	0.029		0.001	0.016

[a]The multivariable Cox regression model initially included sex, age, year of diagnosis, family history of colorectal cancer in parent or sibling, tumor location, disease stage, tumor differentiation, microsatellite instability, CpG island methylator phenotype, *KRAS, BRAF*, and *PIK3CA* mutations, and long interspersed nucleotide element-1 (LINE-1) methylation level. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.
[b]Test for a linear trend was conducted across ordinal quartile categories (1 to4) of tumor *MIR21* expression level as a continuous variable in the Cox regression model.

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Table 3. Tumor *MIR21* expression level and colorectal cancer mortality according to PTGS2 expression

		Colorectal cancer–specific mortality			Overall mortality		
	No. of cases	No. of events	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]	No. of events	Univariable HR (95% CI)	Multivariable HR (95% CI)[a]
PTGS2-absent/low cancer							
MIR21 expression level							
Quartile 1 (lowest)	73	25	1 (reference)	1 (reference)	44	1 (reference)	1 (reference)
Quartile 2	73	14	0.49 (0.26–0.95)	0.57 (0.29–1.11)	33	0.63 (0.40–1.00)	0.71 (0.45–1.12)
Quartile 3	76	26	0.99 (0.57–1.71)	0.90 (0.52–1.57)	43	1.07 (0.70–1.63)	1.01 (0.66–1.56)
Quartile 4 (highest)	73	22	0.91 (0.51–1.62)	0.61 (0.34–1.10)	39	1.04 (0.68–1.61)	0.89 (0.57–1.38)
P_{trend}[b]			0.74	0.22		0.39	0.91
PTGS2-high cancer							
MIR21 expression level							
Quartile 1 (lowest)	119	26	1 (reference)	1 (reference)	60	1 (reference)	1 (reference)
Quartile 2	117	30	1.21 (0.72–2.05)	1.17 (0.69–1.98)	65	1.29 (0.90–1.83)	1.20 (0.84–1.72)
Quartile 3	116	35	1.46 (0.88–2.43)	1.29 (0.77–2.15)	63	1.22 (0.86–1.74)	1.06 (0.74–1.51)
Quartile 4 (highest)	118	53	2.45 (1.53–3.93)	2.28 (1.42–3.67)	82	1.86 (1.33–2.60)	1.75 (1.25–2.45)
P_{trend}[b]			< 0.0001	0.0004		0.0008	0.004
$P_{interaction}$[c]			0.007	0.0004		0.06	0.036

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

[a]The multivariable Cox regression model included sex, age, year of diagnosis, family history of colorectal cancer in parent or sibling, tumor location, disease stage, tumor differentiation, microsatellite instability, CpG island methylator phenotype, *KRAS, BRAF*, and *PIK3CA* mutations, and long interspersed nucleotide element-1 (LINE-1) methylation level. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final models.

[b]Test for a linear trend was conducted across ordinal quartile categories (1 to4) of tumor *MIR21* expression level as a continuous variable in the Cox regression model.

[c]$P_{interaction}$ values (two-sided) were calculated by the Wald test on the cross-product term of tumor *MIR21* expression [ordinal quartile categories (1 to 4)] and PTGS2 expression [ordinal categories; absent (1), low (2), and high (3)] variables in the Cox regression model.

Although the mechanisms underlying the association of tumor *MIR21* expression with *BRAF* mutation in colorectal cancer remain uncertain, experimental evidence suggests that activation of the RAF–MAPK signaling pathway may increase *MIR21* expression level (45), and that *BRAF* mutation may potentiate the STAT3 signaling pathway that has been shown to increase *MIR21* expression level (11, 46). Taken together, *BRAF* mutation might increase *MIR21* expression level through the activation of the MAPK and/or the STAT3 signaling pathways, although additional experimental studies are needed to test this hypothesis. Emerging evidence suggests that PTGS2-derived PGE_2 may suppress antitumor T-cell response, and PTGS2 inhibitors may enhance the efficacy of therapeutic antibodies specific for immune checkpoint molecules in *BRAF*-mutated melanoma (47). Hence, it would be intriguing for future investigations to explore potential influences of tumor *MIR21* and/or PTGS2 expression on the efficacy of the immune checkpoint inhibitors in colorectal cancers.

PTGS2 produces inflammatory mediator PGE_2, which has been shown to promote colorectal tumor progression (3–5). Recent experimental data suggest that inflammatory responses induce *MIR21*, which in turn increases local level of PGE_2 by suppressing degradation of PGE_2 (11–14, 16, 17). These lines of experimental evidence may be consistent with the current population-based data suggesting that the adverse prognostic association of tumor *MIR21* expression level in colorectal cancer is stronger in cancers expressing high-level PTGS2. Experimental evidence also suggests that PTGES (prostaglandin E synthase or microsomal prostaglandin E synthase-1 [mPGES-1]) catalyzes the conversion of prostaglandin H_2 (PGH_2) to PGE_2, and that HPGD [hydroxyprostaglandin dehydrogenase 15-(NAD); or 15-PDGH], SLCO2A1 (solute carrier organic anion transporter family member 2A1 or prostaglandin transporter), and ABCC4 (ATP binding cassette subfamily C member 4 or multidrug resistance-associated protein 4) regulate PGE_2 degradation (3). Hence, additional future studies of tumor expression of HPGD and the other molecules involved in the PGE_2 biosynthetic pathways in relation to *MIR21* expression in colorectal cancer are needed. miRNA-targeting therapies for human disease including cancer are currently being investigated (48). In light of our findings, future investigations may be warranted to explore a potential strategy of inhibiting *MIR21* in treatment for colorectal cancers expressing high-level PTGS2.

We acknowledge limitations of our study. First, data on cancer recurrence were limited in the two cohorts. However, colorectal cancer-specific mortality can be considered as a reasonable cancer-specific outcome in a population-based study with long-term follow-up, because median survival for recurrent (metastatic) colorectal cancer was approximately 10 to 20 months during the time period of this study (49). Second, data on cancer treatment were also limited. However, distributions of chemotherapy use and its regimen would unlikely substantially differ according to tumor *MIR21* and PTGS2 expression in resected specimens, because these data were not available for treatment decisions. We recognize that another limitation of our current study is the lack of a widely accepted, standardized classification scheme for tumor PTGS2 expression levels. We assessed tumor PTGS2 expression by IHC through the central, blinded review of tumor specimens with rigorous comparison with internal controls. The interobserver agreement for tumor PTGS2 expression levels (0.85; $\kappa = 0.69$) was reasonably good. Any random misclassification of tumor PTGS2 expression status would have driven our results towards the null hypothesis. Despite this limitation, we were able to demonstrate the significant interaction between *MIR21* and PTGS2 expression in colorectal cancer mortality analysis.

The strengths of our study include the use of our molecular pathologic epidemiology (50–52) database of rectal and colon carcinoma cases in the two U.S. nationwide, prospective cohort studies, which integrates clinicopathologic features, long-term survival data, and tumor molecular features including miRNA

MIR21 expression in colorectal cancer tissue. This population-based colorectal cancer database enabled us to rigorously examine the interactive prognostic association of tumor *MIR21* and PTGS2, controlling for potential confounders. In addition, our colorectal cancer specimens were derived from a large number of hospitals in diverse settings across the United States, which increase generalizability of our findings.

In conclusion, tumor *MIR21* expression level is associated with higher colorectal cancer mortality independent of clinical, pathologic, and tumor molecular features, and this association is stronger in cancers expressing high-level PTGS2. Additional prospective studies are needed to validate these findings from the current exploratory, hypothesis-generating study. Upon validation, our population-based data may inform future research to develop strategies for colorectal cancer prevention and treatment through targeting *MIR21* and the PTGS2/PGE_2 pathway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors assume full responsibility for analyses and interpretation of these data.

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References

1. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–58.
2. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014;9:287–314.
3. Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer 2010;10:181–93.
4. Wang D, Fu L, Sun H, Guo L, DuBois RN. Prostaglandin E2 promotes colorectal cancer stem cell expansion and metastasis in mice. Gastroenterology 2015;149:1884–95.
5. Ogino S, Kirkner GJ, Nosho K, Irahara N, Kure S, Shima K, et al. Cyclooxygenase-2 expression is an independent predictor of poor prognosis in colon cancer. Clin Cancer Res 2008;14:8221–7.
6. Tougeron D, Sha D, Manthravadi S, Sinicrope FA. Aspirin and colorectal cancer: back to the future. Clin Cancer Res 2014;20:1087–94.
7. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003;348:883–90.
8. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med 2007;356:2131–42.
9. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA 2009;302:649–58.
10. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509–24.
11. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer 2014;14:736–46.
12. Tili E, Michaille JJ, Croce CM. MicroRNAs play a central role in molecular dysfunctions linking inflammation with cancer. Immunol Rev 2013;253:167–84.
13. Shi C, Yang Y, Xia Y, Okugawa Y, Yang J, Liang Y, et al. Novel evidence for an oncogenic role of microRNA-21 in colitis-associated colorectal cancer. Gut. 2015 May 20. [Epub ahead of print].
14. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell 2010;39:493–506.
15. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008;299:425–36.
16. Schetter AJ, Nguyen GH, Bowman ED, Mathe EA, Yuen ST, Hawkes JE, et al. Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. Clin Cancer Res 2009;15:5878–87.
17. Lu L, Byrnes K, Han C, Wang Y, Wu T. miR-21 targets 15-PGDH and promotes cholangiocarcinoma growth. Mol Cancer Res 2014;12:890–900.
18. Nishihara R, Lochhead P, Kuchiba A, Jung S, Yamauchi M, Liao X, et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. JAMA 2013;309:2563–71.
19. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012;367:1596–606.
20. Mima K, Nishihara R, Nowak JA, Kim SA, Song M, Inamura K, et al. MicroRNA *MIR21* and T cells in colorectal cancer. Cancer Immunol Res 2016;4:33–40.

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21. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009;58:90–6.
22. Ogino S, Kawasaki T, Nosho K, Ohnishi M, Suemoto Y, Kirkner GJ, et al. LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer. Int J Cancer 2008;122:2767–73.
23. Irahara N, Nosho K, Baba Y, Shima K, Lindeman NI, Hazra A, et al. Precision of pyrosequencing assay to measure LINE-1 methylation in colon cancer, normal colonic mucosa, and peripheral blood cells. J Mol Diagn 2010;12:177–83.
24. Ogino S, Kawasaki T, Brahmandam M, Cantor M, Kirkner GJ, Spiegelman D, et al. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. J Mol Diagn 2006;8:209–17.
25. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. J Mol Diagn 2007;9:305–14.
26. Ogino S, Kawasaki T, Brahmandam M, Yan L, Cantor M, Namgyal C, et al. Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn 2005;7:413–21.
27. Imamura Y, Lochhead P, Yamauchi M, Kuchiba A, Qian ZR, Liao X, et al. Analyses of clinicopathological, molecular, and prognostic associations of KRAS codon 61 and codon 146 mutations in colorectal cancer: cohort study and literature review. Mol Cancer 2014;13:135.
28. Liao X, Morikawa T, Lochhead P, Imamura Y, Kuchiba A, Yamauchi M, et al. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. Clin Cancer Res 2012;18:2257–68.
29. Weisenberger DJ, Levine AJ, Long TI, Buchanan DD, Walters R, Clendenning M, et al. Association of the colorectal CpG island methylator phenotype with molecular features, risk factors, and family history. Cancer Epidemiol Biomarkers Prev 2015;24:512–9.
30. Campbell PT, Newton CC, Newcomb PA, Phipps AI, Ahnen DJ, Baron JA, et al. Association between body mass index and mortality for colorectal cancer survivors: overall and by tumor molecular phenotype. Cancer Epidemiol Biomarkers Prev 2015;24:1229–38.
31. Tillmans LS, Vierkant RA, Wang AH, Jewel Samadder N, Lynch CF, Anderson KE, et al. Associations between cigarette smoking, hormone therapy, and folate intake with incident colorectal cancer by TP53 protein expression level in a population-based cohort of older women. Cancer Epidemiol Biomarkers Prev 2014;23:350–5.
32. Phipps AI, Ahnen DJ, Cheng I, Newcomb PA, Win AK, Burnett T. PIK3CA Somatic mutation status in relation to patient and tumor factors in racial/ethnic minorities with colorectal cancer. Cancer Epidemiol Biomarkers Prev 2015;24:1046–51.
33. Shiovitz S, Bertagnolli MM, Renfro LA, Nam E, Foster NR, Dzieciatkowski S, et al. CpG island methylator phenotype is associated with response to adjuvant irinotecan-based therapy for stage III colon cancer. Gastroenterology 2014;147:637–45.
34. Park JH, McMillan DC, Powell AG, Richards CH, Horgan PG, Edwards J, et al. Evaluation of a tumor microenvironment-based prognostic score in primary operable colorectal cancer. Clin Cancer Res 2015;21:882–8.
35. Panarelli NC, Vaughn CP, Samowitz WS, Yantiss RK. Sporadic microsatellite instability-high colon cancers rarely display immunohistochemical evidence of Wnt signaling activation. Am J Surg Pathol 2015;39:313–7.
36. Phipps AI, Limburg PJ, Baron JA, Burnett-Hartman AN, Weisenberger DJ, Laird PW, et al. Association between molecular subtypes of colorectal cancer and patient survival. Gastroenterology 2015;148:77–87.
37. Slattery ML, Herrick JS, Mullany LE, Valeri N, Stevens J, Caan BJ, et al. An evaluation and replication of miRNAs with disease stage and colorectal cancer-specific mortality. Int J Cancer 2015;137:428–38.
38. Sinicrope FA, Mahoney MR, Yoon HH, Smyrk TC, Thibodeau SN, Goldberg RM, et al. Analysis of molecular markers by anatomic tumor site in stage III colon carcinomas from adjuvant chemotherapy trial NCCTG N0147 (Alliance). Clin Cancer Res 2015;21:5294–304.
39. Cushman SM, Jiang C, Hatch AJ, Shterev I, Sibley AB, Niedzwiecki D, et al. Gene expression markers of efficacy and resistance to cetuximab treatment in metastatic colorectal cancer: results from CALGB 80203 (Alliance). Clin Cancer Res 2015;21:1078–86.
40. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005;65:6063–9.
41. Ogino S, Shima K, Meyerhardt JA, McCleary NJ, Ng K, Hollis D, et al. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: results from intergroup trial CALGB 89803. Clin Cancer Res 2012;18:890–900.
42. Lochhead P, Kuchiba A, Imamura Y, Liao X, Yamauchi M, Nishihara R, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst 2013;105:1151–6.
43. Seppala TT, Bohm JP, Friman M, Lahtinen L, Vayrynen VM, Liipo TK, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. Br J Cancer 2015;112:1966–75.
44. Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.
45. Huang TH, Wu F, Loeb GB, Hsu R, Heidersbach A, Brincat A, et al. Up-regulation of miR-21 by HER2/neu signaling promotes cell invasion. J Biol Chem 2009;284:18515–24.
46. Becker TM, Boyd SC, Mijatov B, Gowrishankar K, Snoyman S, Pupo GM, et al. Mutant B-RAF-Mcl-1 survival signaling depends on the STAT3 transcription factor. Oncogene 2014;33:1158–66.
47. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell 2015;162:1257–70.
48. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov 2014;13:622–38.
49. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005;352:476–87.
50. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut 2011;60:397–411.
51. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. Mod Pathol 2013;26:465–84.
52. Ogino S, Campbell PT, Nishihara R, Phipps AI, Beck AH, Sherman ME, et al. Proceedings of the second international molecular pathological epidemiology (MPE) meeting. Cancer Causes Control 2015;26:959–72.

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MicroRNA *MIR21* (miR-21) and PTGS2 Expression in Colorectal Cancer and Patient Survival

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