MGMT promoter methylation, loss of expression and prognosis in 855 colorectal cancers

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Abstract

Objective— O^6-methylguanine-DNA methyltransferase (MG MT) is a DNA repair enzyme. *MGMT* promoter hypermethylation and epigenetic silencing often occur as early events in carcinogenesis. However, prognostic significance of *MGMT* alterations in colorectal cancer remains uncertain.

Methods—Utilizing a database of 855 colon and rectal cancers in two prospective cohort studies (the Nurses' Health Study and the Health Professionals Follow-up Study), we detected *MGMT* promoter hypermethylation in 325 tumors (38%) by MethyLight and loss of MGMT expression in 37% (247/672) of tumors by immunohistochemistry. We assessed the CpG island methylator phenotype (CIMP) using eight methylation markers [*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3,* and *SOCS1*], and LINE-1 (L1) hypomethylation, TP53 (p53), and microsatellite instability (MSI).

Results—*MGMT* hypermethylation was not associated with colorectal cancer–specific mortality in univariate or multivariate Cox regression analysis [adjusted hazard ratio (HR) = 1.03; 95% confidence interval (CI), 0.79–1.36] that adjusted for clinical and tumor features, including CIMP, MSI, and *BRAF* mutation. Similarly, MGMT loss was not associated with patient survival. MGMT loss was associated with G>A mutations in *KRAS* ($p = 0.019$) and *PIK3CA* ($p = 0.0031$).

Conclusions—Despite a well-established role of MGMT aberrations in carcinogenesis, neither *MGMT* promoter methylation nor MGMT loss serves as a prognostic biomarker in colorectal cancer.

Keywords

Colon cancer; *MGMT*; Hypermethylation; Epigenetics; Clinical outcome

Introduction

The O^6-methylguanine-DNA methyltransferase (*MGMT*) gene encodes DNA repair protein and is frequently inactivated in colorectal cancer [1, 2]. Polymorphisms in *MGMT* have been associated with colorectal cancer risk [3, 4], and *MGMT* promoter methylation in normal colonic mucosa might be a predisposing factor for cancer as a field effect and an early event in colorectal carcinogenesis [5, 6]. *MGMT* promoter methylation and loss of expression have been associated with G>A mutations in a variety of genes such as *KRAS*, *PIK3CA*, *TP53,* and *APC* [7–11]. Jass [12] proposed the molecular classification based on CIMP, MSI, *BRAF*, *KRAS*, and *MGMT* promoter methylation, indicating that *MGMT* methylation is one of the key molecular alterations in colorectal cancer. In addition, *MGMT* has potential as a therapeutic target in human cancer [13, 14]. Collectively, it is of interest to examine a prognostic role of *MGMT* alteration as a tumor biomarker. In brain tumors and B-cell lymphoma, *MGMT* methylation or loss of MGMT has been associated with poor prognosis [15–17]. However, prognostic significance of *MGMT* alteration in colorectal cancer remains inconclusive due to limited statistical power of all previous studies (Table 1; all $n < 200$) [18–22].

In this study using the database of a large number ($n = 855$) of stage I–IV colorectal cancers, we examined the prognostic effect of *MGMT* promoter methylation and loss of expression. Since we concurrently assessed other molecular variables including LINE-1 hypomethylation, MSI, CIMP, and mutation in *KRAS*, *BRAF*, and *PIK3CA*, we could evaluate the prognostic effect of *MGMT* alteration after controlling for those potential confounders.

Materials and methods

Study population

We utilized the database of two prospective cohort studies, the Nurses' Health Study (n = 121,701 women followed since 1976) [23] and the Health Professionals Follow-up Study (n = 51,529 men followed since 1986) [23]. Participants have been sent biennial questionnaires to update information on potential risk factors and to identify newly diagnosed cancers in themselves and their first-degree relatives. We collected paraffin-embedded tumor tissue blocks of incident colorectal cancers from hospitals where participants with colorectal cancer underwent tumor resection. Hematoxylin and eosin (H&E)–stained tissue sections from all colorectal cancer cases were confirmed by a pathologist (S.O.) unaware of other data. The tumor grade was categorized as low versus high (>50 vs. ≤50% gland formation). Positive family history of colorectal cancer was defined as the presence of colorectal cancer in any first-degree relative. We excluded cases that were preoperatively treated. Based on the availability of adequate follow-up and tumor tissue data, 855 stage I–IV colorectal cancer cases diagnosed up to 2002 were included. Patients were observed until death or June 2009, whichever came first. This study was approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

Pyrosequencing of *KRAS*, *BRAF,* and *PIK3CA*, and microsatellite instability (MSI) analysis

Genomic DNA was extracted from paraffin-embedded tissue. PCR and pyrosequencing targeted for *KRAS* (codons 12 and 13) [24], *BRAF* (codon 600) [25], and *PIK3CA* (exons 9 and 20) [11] were performed. Microsatellite instability (MSI) analysis was performed using 10 micro-satellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) [26]. MSI-high was defined as the presence of instability in ≥30% of the markers and MSI-low/microsatellite stable (MSS) as instability in 0–29% of markers [26].

Methylation analyses for CpG islands and LINE-1

Sodium bisulfite treatment and subsequent real-time PCR were performed to quantify promoter methylation in *MGMT* and eight other CpG islands (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3,* and *SOCS1*) [27, 28]; the latter eight markers have been shown to be specific for CIMP [29]. CIMP-high was defined as the presence of ≥6/8 methylated markers, CIMP-low as the presence of 1/8–5/8 methylated markers, and CIMP-0 as the absence (0/8) of methylated markers [30]. We defined and validated the cut point for *MGMT* promoter methylation positivity (percentage of methylated reference, or PMR> 4) as previously described [31]. LINE-1 methylation levels were quantified by PCR-pyrosequencing [32, 33].

Immunohistochemical analysis

Immunohistochemical methods for MGMT and TP53 (p53) were previously described [26], and expression patterns were interpreted by a pathologist (S.O.) unaware of other data. In agreement studies, a random selection of more than 100 cases for each marker was interpreted by a second pathologist unaware of other data (MGMT by K.S.; TP53 by K.N.). The concordance between the two observers (both $p < 0.0001$) was 0.86 for MGMT (κ = 0.70) and 0.87 for TP53 (κ = 0.75), indicating substantial agreement. The concordance between *MGMT* methylation and loss of MGMT was 81% (κ = 0.59).

Statistical analysis

We used SAS program (Version 9.1, SAS Institute, Cary, NC) for all statistical analysis. All p values were two-sided, and significance level was set at $p = 0.05$. The chi-square test (or

Fisher's exact test) was performed for categorical variables. For survival analysis, the Kaplan–Meier method and log-rank test were used. For analyses of colorectal cancer–specific mortality, deaths as a result of causes other than colorectal cancer were censored. To control for confounding, we used multivariate stage-matched (stratified) Cox proportional hazard models to compute hazard ratio (HR) of death according to *MGMT* status. To avoid residual confounding and overfitting, disease stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown) was used as a stratifying variable, utilizing the "strata" option in the SAS "proc phreg" command. The multivariate model initially included age at diagnosis (continuous), sex, year of diagnosis (continuous), BMI (<30 vs. ≥30 kg/m^2), family history of colorectal cancer (present vs. absent), tumor location (proximal colon vs. distal colon vs. rectum), tumor grade (low vs. high), MSI (high vs. low/MSS), CIMP (high vs. low vs. CIMP-0), LINE-1 methylation (continuous), and *BRAF*. A backward elimination with a threshold of $p = 0.20$ was used to select variables in the final model. For cases with missing information in any of the categorical variables [tumor location (0.9%), tumor grade (0.5%), MSI (3.0%), *BRAF* (4.8%)], we included those cases in a majority category of a given covariate to avoid overfitting. We confirmed that excluding cases with missing information in any of the covariates did not substantially alter results (data not shown). An interaction was assessed by including the cross product of the *MGMT* methylation (or MGMT loss) variable and another variable of interest (without data-missing cases) in a multivariate model, and the Wald test was performed. A p value for statistical significance was adjusted for multiple hypothesis testing to $p = 0.0029$ (=0.05/17) by Bonferroni correction.

In addition, we constructed multivariate logistic regression analysis model to assess the independent effect of MGMT loss on G>A mutations in *KRAS* or *PIK3CA* (as a binary outcome variable). The model initially included age at diagnosis (continuous), sex, year of diagnosis (continuous), BMI (<30 vs. ≥30 kg/m^2), family history of colorectal cancer (present vs. absent), tumor location (proximal vs. distal), MSI (high vs. low/MSS), CIMP (high vs. low/0), LINE-1 methylation (continuous), and MGMT loss, and a backward elimination with a threshold of $p = 0.20$ was done to select variables in the final model.

Results

MGMT methylation and loss of MGMT in colorectal cancer

MGMT promoter methylation was detected in 325 (38%) of 885 tumors, and loss of MGMT was detected in 247 (37%) of 672 tumors. There was a significant association between *MGMT* promoter methylation and CIMP status ($p < 0.0001$). Loss of MGMT was significantly associated with *PIK3CA* mutation ($p = 0.0031$) and inversely associated with TP53 expression ($p = 0.0004$; Table 2).

MGMT methylation/loss and G>A mutations in *KRAS* and *PIK3CA*

Because functional loss of *MGMT* may contribute to G>A mutations [7–11], we examined the relations between *MGMT* methylation (or loss) and G>A mutations in *KRAS* and *PIK3CA* (Table 2). MGMT loss was significantly associated with G>A mutations in *KRAS* ($p = 0.019$) and *PIK3CA* ($p = 0.0031$) (by a priori hypothesis testing), while *MGMT* methylation was not. In multivariate logistic regression analysis to assess independent effect of MGMT loss on G>A mutations, MGMT loss remained significantly associated with G>A mutations in *KRAS* (adjusted OR, 1.58; 95% CI, 1.07–2.34; $p = 0.021$) and *PIK3CA* (adjusted OR, 2.55; 95% CI, 1.40–4.68; $p = 0.0024$).

MGMT methylation, loss of MGMT, and survival of patients with colorectal cancer

Among the 855 patients, there were 415 deaths including 234 colorectal cancer–specific deaths. The median follow-up time for censored patients was 13.0 years. For either

colorectal cancer–specific or overall mortality, *MGMT* methylation was not significantly associated with patient outcome in log-rank test, or univariate or multivariate stage–matched Cox regression analysis (Table 3, Fig. 1). Likewise, loss of MGMT was not significantly associated with colorectal cancer–specific or overall mortality in univariate or multivariate stage–matched analysis (Table 3, Fig. 1).

We analyzed the prognostic effect of *MGMT* methylation or loss of MGMT in colon cancer and rectal cancer separately, since clinical management for patients with colon cancer differ from that for patients with rectal cancer. *MGMT* methylation (or MGMT loss) was not significantly associated with colorectal cancer–specific or overall mortality in either patients with colon cancer or rectal cancer (Table 3).

There was no significant modifying effect on the prognostic influence of *MGMT* methylation (or MGMT loss) by Table 2 Clinical and molecular characteristics in colorectal cancer with *MGMT* promoter methylation/loss of MGMT any of the other variables including sex, age, year of diagnosis, BMI, family history of colorectal cancer, tumor location, stage, tumor grade, CIMP, MSI, *BRAF*, *KRAS*, *PIK3CA*, LINE-1 methylation, and TP53 (all $P_{\text{interaction}} > 0.02$).

Discussion

We conducted this study to examine whether *MGMT* promoter methylation or loss of expression in colorectal cancer has any prognostic role. This question has remained inconclusive due to limited statistical power of all previous studies on this topic [18–22]. Given the crucial roles of *MGMT* aberrations in colorectal carcinogenesis or a potential use of *MGMT* as a therapeutic target in human cancer, the assessment of *MGMT* alteration (i.e., *MGMT* promoter methylation or loss of MGMT) and clinical outcome using a large number of cancers is needed. Utilizing the database of 855 clinically and molecularly annotated colorectal cancers in the two large prospective cohort studies, we found that *MGMT* alteration was not associated with patient prognosis in colorectal cancer. In addition, we assessed the prognostic effect of *MGMT* promoter methylation (or MGMT loss), controlling for other molecular features, including CIMP, MSI, and *BRAF* mutation, all of which have been documented to be critical in colorectal carcinogenesis.

Studying molecular variants and somatic changes is important in cancer research [34–42]. Recent studies have shown that *MGMT* promoter polymorphism (rs16906252) is associated with *MGMT* methylation in colorectal cancer [43], in normal colorectal mucosa [44], and in peripheral blood cells from normal individuals [45]. Epigenetic silencing of *MGMT* by promoter methylation in normal colonic mucosa may be a predisposing factor for cancer as a field effect and an early event in colorectal carcinogenesis [5, 6]. In addition, *MGMT* methylation might be a valuable biomarker in plasma for early detection of colorectal cancer [46].

Studies on colorectal cancer have shown that *MGMT* methylation is associated with MGMT loss [5, 6, 47]. In agreement with these studies [5, 6, 47], our current study showed a good concordance (81%, $\kappa = 0.59$, $p < 0.0001$) between *MGMT* methylation and MGMT loss. *MGMT* methylation and loss of MGMT were not perfectly correlated perhaps due to a few reasons. First, loss of MGMT expression may be caused not only by promoter methylation but also by other mechanisms such as a gene deletion or mutation. Second, in rare cases, promoter methylation may be present in only one *MGMT* allele, and the MGMT protein may be expressed from the second allele. Third, there may be other molecules (such as miRNA) that may downregulate *MGMT*.

MGMT promoter methylation or loss of expression in colorectal cancer has been associated with G>A mutations in *KRAS* [7, 9, 10, 44, 48], *TP53* [8, 9], and *PIK3CA* [11]. Our current study is the first one to perform multivariate analysis and show that MGMT loss is associated with G>A mutations in *KRAS* and *PIK3CA*, independent of potential confounders. Thus, our current study supports the concept that loss of MGMT contributes to G>A mutations of *KRAS* and *PIK3CA*.

Previous studies [18–22] have shown no prognostic significance of *MGMT* methylation (or loss of MGMT) in colorectal cancers (Table 1). These previous studies [18–22] on prognostic significance of *MGMT* methylation (or MGMT loss) are limited by low statistical power ($n < 200$). In contrast to the prior studies [18–22], our study examined both *MGMT* promoter methylation and loss of MGMT expression in a much larger cohort of colorectal cancers. In the current study, *MGMT* methylation was found in 38% of colorectal cancer. Previous studies [18–22] have shown a large variation in the frequency of *MGMT* promoter methylation (21–61%; Table 1). This variation might be caused by differences in study samples and/or methylation detection methods (MSP vs. quantitative MethyLight vs. Pyrosequencing) and might also be in part due to a chance variation in the small studies.

With regard to the predictive role of MGMT aberrations, Braun et al. [34] examined a predictive role of loss of MGMT expression (among other markers) in 1,125 patients with metastatic colorectal cancer who underwent different chemotherapy treatment arms (fluorouracil vs. fluorouracil/irinotecan vs. fluorouracil/oxaliplatin) and found no predictive role of MGMT aberrations.

There are limitations in this study. For example, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use substantially differed according to *MGMT* status in tumor, since such data were unavailable for treatment decision making. In addition, our multivariate survival analysis adjusted for disease stage as finely as I, IIA, IIB, IIIA, IIIB, IIIC, IV on which treatment decision making was mostly based. As another limitation, beyond cause of mortality, data on cancer recurrences were unavailable in these cohort studies. Nonetheless, colorectal cancer–specific survival might be a reasonable surrogate of colorectal cancer–specific outcome.

There are advantages in utilizing the database of the two prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-up Study, to examine prognostic significance of tumor biomarkers. Anthropometric measurements, family history, cancer staging, and other clinical, pathologic, and tumoral molecular data were prospectively collected, blinded to patient outcome. Cohort participants who developed cancer were treated at hospitals throughout the United States and thus more representative colorectal cancers in the US population than patients in one to a few academic hospitals. There were no demographic difference between cases with tumor tissue analyzed and those without tumor tissue analyzed [23]. Finally, our rich tumor database enabled us to simultaneously assess pathologic and tumoral molecular correlates and control for confounding by a number of tumoral molecular alterations.

In conclusion, our findings suggest that *MGMT* promoter methylation or loss of expression is not a prognostic biomarker in colorectal cancer, despite its well-established role in carcinogenesis.

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Abbreviations

BMI	Body mass index
CI	Confidence interval
CIMP	CpG island methylator phenotype
HR	Hazard ratio
MGMT	O^6-methylguanine-DNA methyltransferase
MSI	Microsatellite instability
MSS	Microsatellite stable
OR	Odds ratio

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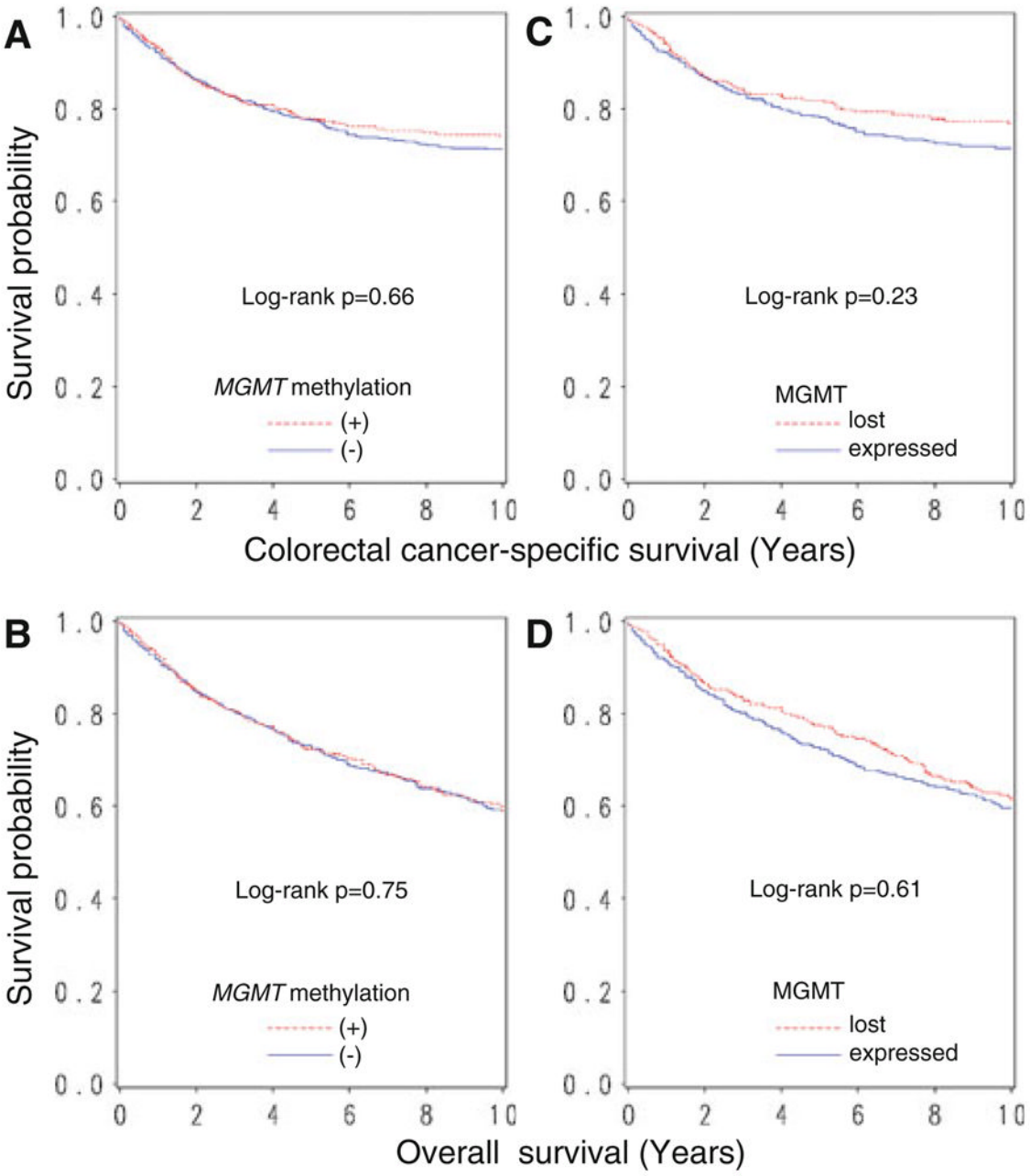

Fig. 1.

Kaplan–Meier curves for colorectal cancer–specific survival (*upper panel*) and overall survival (*lower panel*), according to *MGMT* promoter methylation (**a**, **b**) or loss of MGMT (**c**, **d**) in colorectal cancer

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Table 1

Studies on prognostic significance of *MGMT* methylation (top) or loss of MGMT (bottom) in colorectal cancer

Ref.	Authors (year)	Sample size	No. of events		Disease stage	Chemotherapy	*MGMT* methylation (vs. no methylation) or loss of MGMT (vs. expression)						
			OS	CS			Method	Methylated (MGMT-lost) cases (%)	*p* value by log-rank test	OS univariate HR (95% CI)	*p* value by univariate model	OS multi-variate HR (95% CI)	*p* value by multi-variate model	Note
MGMT methylation														
[18]	Nagasaka et al. (2003)	90	–	–	Dukes A-D	Fluoro-pyrimidines	MSP	29	–	–	–	1.30[a] (0.27–6.47)	NS	
[19]	Krtolica et al. (2007)	47	–	–	Dukes A-D	–	MSP	43	NS	–	NS	–	NS	
[20]	Chen et al. (2009)	117	–	–	Dukes A-D	Adjuvant chemotherapy	MSP	61	–	–	–	1.33 (0.61–2.92)	$p = 0.47$	
[21]	Kim et al. (2010)	131	–	–	I–IV	Adjuvant chemotherapy	Pyro-sequencing	21	–	–	NS		NS	Rectal cancer only
	Current study	855	415	234	I–IV	–	q-MSP	38	$p = 0.83$	1.03 (0.85–1.26)	$p = 0.75$	1.08 (0.88–1.32)	$p = 0.49$	
Loss of MGMT														
[22]	Kohonen-Corish et al. (2005)	178	141	–	Dukes C	Adjuvant chemotherapy	IHC	22	$p = 0.55$	–	–	–	–	
	Current study	672	313	170	I–IV	–	IHC	37	$p = 0.54$	0.94 (0.75–1.18)	$p = 0.59$	1.11 (0.87–1.41)	$p = 0.39$	

CI confidence interval, *CIMP* CpG island methylator phenotype, *CS* colorectal cancer–specific survival, HR, hazard ratio, *IHC* immunohistochemistry, *MSP* methylation-specific PCR, *NS* not significant, *OS* overall survival, *q-MSP* quantitative methylation–specific PCR

[a] Patients with unmethylated *MGMT* promoters at a 1.3-fold increased risk of death

Table 2

Clinical and molecular characteristics in colorectal cancer with *MGMT* promoter methylation/loss of MGMT

Clinical or molecular feature	All cases	*MGMT* promoter methylation			MGMT status		
		(−)	(+)	*p* value	Expressed	Lost	*p* value
Total *n*	855	530	325		425	247	
Sex				0.23			0.71
Male (HPFS)	367 (43%)	236 (45%)	131 (40%)		173 (41%)	97 (39%)	
Female (NHS)	488 (57%)	294 (55%)	194 (60%)		252 (59%)	150 (61%)	
Mean age ± SD	66.2 ± 8.4	66.2 ± 8.6	66.3 ± 8.1	0.72	66.3 ± 8.4	65.9 ± 7.9	0.60
Body mass index (BMI)				0.38			0.093
<30 kg/m^2	714 (83%)	438 (83%)	276 (85%)		347 (82%)	214 (87%)	
≥30 kg/m^2	141 (17%)	92 (17%)	49 (15%)		78 (18%)	33 (13%)	
Family history of colorectal cancer in any 1st degree relative				0.47			0.28
Absent	646 (76%)	396 (75%)	250 (77%)		319 (75%)	176 (71%)	
Present	209 (24%)	134 (25%)	75 (23%)		106 (25%)	71 (29%)	
Year of diagnosis				0.68			0.031
Prior to 1995	400 (47%)	245 (46%)	155(48%)		182 (43%)	127 (51%)	
1995–2004	455 (53%)	285 (54%)	170 (52%)		243 (57%)	120 (49%)	
Tumor location				0.90			0.014
Proximal colon (cecum to transverse)	381 (45%)	239 (46%)	142 (44%)		197 (47%)	108 (44%)	
Distal colon (splenic flexure to sigmoid)	273 (32%)	168 (32%)	105 (33%)		125 (30%)	96 (39%)	
Rectum	192 (23%)	117 (22%)	75 (23%)		100 (24%)	40 (16%)	
Disease stage				0.86			0.70
I	199 (23%)	124 (23%)	75 (23%)		94 (22%)	57 (23%)	
II	259 (30%)	156 (29%)	103 (32%)		133 (31%)	77 (31%)	
III	228 (27%)	146 (28%)	82 (25%)		110 (26%)	69 (28%)	
IV	102 (12%)	65 (12%)	37 (11%)		57 (13%)	24 (9.7%)	
Unknown	67 (7.8%)	39 (7.4%)	28 (8.6%)		31 (7.3%)	20 (8.1%)	
Tumor grade				0.63			0.23
Low	771 (91%)	480 (91%)	291 (90%)		389 (92%)	220 (89%)	
High	79 (9.3%)	47 (8.9%)	32 (9.9%)		33 (7.8%)	26 (11%)	

Clinical or molecular feature	All cases	*MGMT* promoter methylation			MGMT status		
		(−)	(+)	*p* value	Expressed	Lost	*p* value
MSI status				0.19			0.21
MSS/MSI-low	705 (85%)	442 (87%)	263 (83%)		361 (86%)	201 (83%)	
MSI-high	124 (15%)	70 (13%)	54 (17%)		57 (14%)	42 (17%)	
CIMP status				<0.0001			0.0077
CIMP-0	399 (47%)	280 (53%)	119 (37%)		218 (51%)	96 (39%)	
CIMP-low	325 (38%)	184 (35%)	141 (43%)		145 (34%)	107 (43%)	
CIMP-high	131 (15%)	66 (12%)	65 (20%)		62 (15%)	44 (18%)	
KRAS mutation				0.75			0.019
(−)	529 (63%)	332 (64%)	197 (62%)		281 (67%)	136 (56%)	
Non G>A mutation	185 (22%)	111 (22%)	74 (23%)		86 (20%)	66 (27%)	
G>A	121 (14%)	73 (14%)	48 (15%)		53 (13%)	41 (17%)	
BRAF mutation				0.92			0.11
(−)	703 (87%)	434 (86%)	269 (86%)		347 (84%)	209 (89%)	
(+)	111 (13%)	68 (14%)	43 (14%)		64 (16%)	26 (11%)	
PIK3CA mutation				0.079			0.0031
(−)	627 (84%)	397 (86%)	230 (81%)		335 (88%)	167 (79%)	
Non G>A mutation	53 (7.1%)	26 (5.7%)	27 (9.5%)		23 (6.1%)	16 (7.6%)	
G>A	64 (8.6%)	36 (7.8%)	28 (9.8%)		21 (5.5%)	28 (13%)	
Mean LINE-1 methylation (%) ± SD	61.4 ± 9.5	61.3 ± 9.8	61.6 ± 9.0	0.70	60.9 ± 9.4	61.2 ± 8.8	0.63
TP53 expression				0.60			0.0004
(−)	482 (57%)	295 (56%)	187 (58%)		219 (52%)	160 (66%)	
(+)	362 (43%)	228 (44%)	134 (42%)		206 (48%)	84 (34%)	

CIMP CpG island methylator phenotype, *CRC* colorectal cancer, *HPFS* Health Professionals Follow-up Study, *MSI* microsatellite instability, *MSS* microsatellite stable, *NHS* Nurses' Health Study, *SD* standard deviation

Table 3

MGMT promoter methylation/loss of MGMT in colorectal cancer and patient mortality

	Total *n*	Colorectal cancer–specific mortality			Overall mortality		
		Deaths/person-years	Univariate HR (95% CI)	Multivariate HR (95% CI)	Deaths/person-years	Univariate HR (95% CI)	Multivariate HR (95% CI)
Colorectal cancer							
MGMT promoter							
Unmethylated	530	148/4,996	1 (referent)	1 (referent)	256/4,996	1 (referent)	1 (referent)
Methylated	325	86/3,002	0.95 (0.73–1.24)	1.03 (0.79–1.36)	159/3,002	1.03 (0.85–1.26)	1.08 (0.88–1.32)
MGMT							
Expressed	425	116/3,930	1 (referent)	1 (referent)	200/3,930	1 (referent)	1 (referent)
Lost	247	58/2,450	0.83 (0.61–1.14)	1.08 (0.85–1.37)	116/2,450	0.94 (0.75–1.18)	1.11 (0.87–1.41)
Colon cancer							
MGMT promoter							
Unmethylated	407	115/3,807	1 (referent)	1 (referent)	199/3,832	1 (referent)	1 (referent)
Methylated	247	57/2,336	0.80 (0.58–1.09)	0.85 (0.61–1.18)	118/2,354	0.96 (0.77–1.21)	1.00 (0.79–1.26)
MGMT							
Expressed	322	87/2,993	1 (referent)	1 (referent)	151/2,993	1 (referent)	1 (referent)
Lost	204	47/2,020	0.82 (0.58–1.17)	1.03 (0.71–1.49)	97/2,020	0.96 (0.74–1.24)	1.12 (0.86–1.46)
Rectal cancer							
MGMT promoter							
Unmethylated	117	31/1,129	1 (referent)	1 (referent)	53/1,129	1 (referent)	1 (referent)
Methylated	75	27/652	1.47 (0.87–2.46)	1.37 (0.79–2.36)	38/652	1.24 (0.82–1.89)	1.17 (0.75–1.84)
MGMT							
Expressed	100	27/920	1 (referent)	1 (referent)	46/920	1 (referent)	1 (referent)
Lost	40	9/416	0.77 (0.36–1.64)	0.96 (0.41–2.27)	16/416	0.77 (0.44–1.36)	1.05 (0.57–1.93)

The multivariate, stage-matched (stratified) Cox regression model initially included the *MGMT* promoter methylation or loss of MGMT variable, sex, age at diagnosis, year of diagnosis, tumor location, obesity, family history of colorectal cancer, tumor grade, CIMP, MSI, *BRAF*, and LINE-1 methylation. A backward elimination with threshold of $p = 0.20$ was used to select variables in the final models. *CI* confidence interval, *HR* hazard ratio