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Prognostic Significance and Molecular Features of Signet-Ring Cell and Mucinous Components in Colorectal Carcinoma

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Abstract

Background—Colorectal carcinoma (CRC) represents a group of histopathologically and molecularly heterogeneous diseases, which may contain signet-ring cell component and/or mucinous component to a varying extent under pathology assessment. However, little is known about the prognostic significance of those components, independent of various tumor molecular features.

Methods—Utilizing a molecular pathological epidemiology database of 1,336 rectal and colon cancers in the Nurses' Health Study and the Health Professionals Follow-up Study, we examined patient survival according to the proportion of signet-ring cell and mucinous components in CRCs.

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Use of Standardized Official Symbols We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including BRAF; KRAS; PIK3CA; all of which are described at www.genenames.org.

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Cox proportional hazards models were used to compute hazard ratio (HR) for mortality, adjusting for potential confounders including stage, microsatellite instability, CpG island methylator phenotype, LINE-1 methylation, and *KRAS*, *BRAF*, and *PIK3CA* mutations.

Results—Compared to CRC without signet-ring cell component, 1–50 % signet-ring cell component was associated with multivariate CRC-specific mortality HR of 1.40 [95 % confidence interval (CI) 1.02–1.93], and >50 % signet-ring cell component was associated with multivariate CRC-specific mortality HR of 4.53 (95 % CI 2.53–8.12) ($P_{\text{trend}} > 0.0001$). Compared to CRC without mucinous component, neither 1–50 % mucinous component (multivariate HR 1.04; 95 % CI 0.81–1.33) nor >50 % mucinous component (multivariate HR 0.82; 95 % CI 0.54–1.23) was significantly associated with CRC-specific mortality ($P_{\text{trend}} < 0.57$).

Conclusions—Even a minor (50 % or less) signet-ring cell component in CRC was associated with higher patient mortality, independent of various tumor molecular and other clinicopathological features. In contrast, mucinous component was not associated with mortality in CRC patients.

Colorectal carcinoma (CRC) represents a group of histopathologically heterogeneous tumors, with varying amounts of signet-ring cell and/or mucinous component. Signet-ring cell component is characterized by intracytoplasmic mucin displacing the nucleus aside. Mucinous component is characterized by mucin pool in extracellular space. Signet-ring cell carcinoma or mucinous carcinoma (defined as carcinoma with more than 50 % of signet-ring cell or mucinous component, respectively) constitutes approximately 1 or 5–15 % of CRC cases, respectively.^{1–11}

Signet-ring cell carcinoma and mucinous carcinoma have been associated with specific molecular features, including high-degree microsatellite instability (MSI-high), high-frequency of CpG island methylator phenotype (CIMP-high), higher methylation level of long interspersed nucleotide element-1 (LINE-1), and frequent *BRAF* mutation.^{8,12–16} However, molecular features of CRC with 50 % or less of signet-ring cell or mucinous component are yet to be fully characterized.¹²

Although signet-ring cell carcinoma has been associated with shorter patient survival, the prognostic association of mucinous carcinoma remains unclear with studies reporting variable results (Supplementary Table 1).^{2–6} Although many previous studies examining prognostic association of signet-ring cell or mucinous component are available, most of them have been limited by their inability to control for key molecular features of CRC, such as MSI, CIMP, LINE-1 methylation, and *BRAF* mutation, all of which are potential confounders.^{17–24} Moreover, prognostic significance of the presence of 50 % or less of signet-ring cell or mucinous component remains uncertain. We hypothesized that the presence of 50 % or less of signet-ring cell or mucinous component might be associated with shorter CRC patient survival, independent of multiple molecular characteristics and other clinicopathological features.

To test this hypothesis, we utilized a molecular pathological epidemiology^{25,26} database of 1,336 CRC cases in two U.S. nationwide prospective cohort studies, and examined patient survival according to the proportion of signet-ring cell and mucinous components, adjusting

for potential confounders including MSI, CIMP, LINE-1 methylation, and *KRAS*, *BRAF*, and *PIK3CA* mutations.

MATERIALS AND METHODS

Study Population

We utilized the database of two prospective cohort studies: the Nurses' Health Study (NHS, $N = 121,700$ women followed since 1976) and the Health Professionals Follow-up Study (HPFS, $N = 51,529$ men followed since 1986).^{27,28} Participants were sent follow-up biennial questionnaires to update information on potential disease risk factors, and to identify newly diagnosed cancers and other diseases in themselves and their first-degree relatives.

Lethal CRC cases were identified and confirmed through the National Death Index. Formalin-fixed paraffin-embedded tissue blocks were collected from hospitals where participants with CRC had undergone tumor resection. According to the colorectal continuum model, we combined colon and rectal carcinomas.^{29,30} On the basis of the availability of tissue specimens for pathological analyses and survival data, we included a total of 1,336 CRC cases (37 %) diagnosed up to 2008, from 3,593 CRC cases which were observed in the two cohorts. Patients were observed until death or January 1, 2012, whichever came first. The causes of death were assigned by study physicians. Informed consent was obtained from all study participants. This study was approved by the institutional review board for the Harvard School of Public Health and the Brigham and Women's Hospital (IRB approval number: 1999-P-011117).

Histopathological Evaluation

Hematoxylin and eosin-stained tissue sections from all CRC cases were evaluated by a pathologist (S.O.) unaware of other data. Tumor differentiation was categorized as well to moderate versus poor (>50 vs. ≤50 % glandular area).¹ The proportions of signet-ring cell component and mucinous component were recorded as percentage and categorized as 0, 1 to 50, or >50 % of the tumor volume. The cutoff value of 50 % of signet-ring cell or mucinous component was used based on the WHO Classification of Tumours of the Digestive System, which defines signet-ring cell carcinoma or mucinous carcinoma as carcinoma with more than 50 % of signet-ring cell or mucinous component, respectively.¹ Any CRC can have signet-ring cell or mucinous component to any extent (from 0 to 100 %). Any CRC can have both signet-ring cell component and mucinous component, either one of them, or neither of them.

A random selection of more than 150 cases was reviewed for each component by a second pathologist (K.I.) unaware of other data. The concordance between two observers (all $P < 0.0001$) was Spearman $r = 0.83$ for signet-ring cell component (continuous), weighted $\kappa = 0.83$ for signet-ring cell component (trichotomized as 0 vs. 1–50 vs. >50 %), Spearman $r = 0.92$ for mucinous component (continuous), weighted $\kappa = 0.81$ for mucinous component (trichotomized as 0 vs. 1–50 vs. >50 %), indicating substantial agreement.

Analyses of KRAS, BRAF, PIK3CA, and MSI

DNA was extracted from archival tumor tissue.³¹ Polymerase chain reaction (PCR) and pyrosequencing targeted for *KRAS* (codons 12 and 13), *BRAF* (codon 600), and *PIK3CA* (exons 9 and 20) were performed as previously described.^{32–35} MSI analysis was performed utilizing 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) as previously described.¹² We defined MSI-high as the presence of instability in ≥30 % of the markers, and microsatellite stable (MSS) as instability in <30 % of the markers.

Methylation Analysis of CpG Islands and LINE-1

Bisulfite treatment on genomic DNA and subsequent real-time PCR (MethyLight) were validated and performed as previously described.³⁶ We quantified DNA methylation in eight CIMP-specific promoters (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*) as previously described.^{19,37} CIMP-high was defined as the presence of 6/8 or more methylated promoters, and CIMP-low/negative as 0/8 to 5/8 methylated promoters, according to previously established criteria.¹⁹ To quantify LINE-1 methylation level, we performed bisulfite DNA treatment, PCR, and pyrosequencing, after assay validation.³⁸

Statistical Analysis

All statistical analyses were conducted using SAS software (version 9.3, SAS Institute, Cary, NC). All *P* values were two-sided and statistical significance was set at *P* = 0.05. Our primary hypothesis was that the presence of 50 % or less of signet-ring cell or mucinous component might be associated with higher CRC-specific mortality. For secondary analyses, we recognized multiple comparisons inherent in subgroup analyses and interpreted our data very cautiously to avoid over-interpretation. The Mantel–Haenszel Chi square test was performed to test for differences in the distribution of ordinal categorical data. *P* values were calculated by analysis of variance for age and LINE-1 methylation level.

Kaplan–Meier method and log-rank test were used for survival analyses. For analyses of CRC-specific mortality, deaths as a result of other causes were censored. To control for confounding, we used multivariate Cox proportional hazards regression models. In addition to signet-ring cell component variable (0, 1–50, >50 %; ordinal) or mucinous component variable (0, 1–50, >50 %; ordinal), the following covariates were initially included in the regression models; sex, age at diagnosis (continuous), year of diagnosis (continuous), family history of CRC in first-degree relative or relatives (present vs. absent), tumor location (proximal colon vs. distal colon vs. rectum), tumor differentiation (well-moderate vs. poor; only for analyses of mucinous component), MSI (high vs. MSS), CIMP (high vs. low/negative), LINE-1 methylation (continuous), *KRAS*, *BRAF*, and *PIK3CA* mutations. Disease stage (I, II, III, IV, or unknown) was used as a stratifying variable using the “strata” option in the SAS “proc phreg” command to minimize residual confounding. A backward stepwise elimination was carried out with *P* = 0.05 as a threshold, to select variables for the final model. For cases with missing information in any of the categorical covariates [family history of CRC (0.4 %), tumor location (1.0 %), tumor differentiation (0.9 %), MSI (9.8 %), CIMP (13 %), *KRAS* (8.7 %), *BRAF* (8.8 %), and *PIK3CA* (15 %)], we included those cases

in the majority category of a given covariate, and the results were essentially unchanged by excluding cases with missing information on these variables (data not shown). Interaction was assessed by the Wald test on interaction terms that were the cross-products of the variables of interest, and statistical significance level was corrected for multiple hypothesis testing to $P = 0.004$ ($=0.05/13$). The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross products of signet-ring cell component variable/mucinous component variable and survival time (all $P > 0.07$).

RESULTS

Signet-Ring Cell/Mucinous Component in CRC

Table 1 shows characteristics of the 1,336 cases of CRC in the two cohort studies, according to the proportion (0, 1–50, or >50 %) of signet-ring cell or mucinous component. Higher proportion of signet-ring cell and mucinous components were both associated with proximal tumor location, MSI-high, CIMP-high, MLH1 promoter hyper-methylation, frequent *BRAF* mutation, and higher LINE-1 methylation level (all $P < 0.0001$).

Signet-Ring Cell/Mucinous Component and Mortality in CRC

Among the 1,336 patients, there were 698 deaths, including 384 CRC-specific deaths, during a median follow-up of 152 months (interquartile range 112–209 months) for censored cases. Compared to CRC without signet-ring cell component, CRC with signet-ring cell component was associated with higher CRC-specific mortality in Kaplan–Meier analysis (log-rank $P < 0.0001$; Fig. 1), and in univariate and multivariate Cox regression analyses [for 1–50 % signet-ring cell component; multivariate hazard ratio (HR), 1.40; 95 % confidence interval (CI), 1.02–1.93; for >50 % signet-ring cell component; multivariate HR 4.53; 95 % CI 2.53–8.12; $P_{\text{trend}} < 0.0001$; Table 2]. Compared to CRC without mucinous component, CRC with mucinous component was not associated with CRC-specific mortality in Kaplan–Meier analysis (log-rank $P = 0.56$; Fig. 2), and in univariate and multivariate Cox regression analyses [for 1–50 % mucinous component; multivariate HR 1.04; 95 % CI 0.81–1.33; for >50 % mucinous component; multivariate HR 0.82; 95 % CI 0.54–1.23; $P_{\text{trend}} = 0.57$; Table 2].

We also used signet-ring cell/mucinous component as a continuous variable in secondary analyses. Higher CRC-specific mortality was associated with increasing signet-ring cell component (for unit increase of 10 %; multivariate HR 1.16; 95 % CI 1.09–1.24), but not with increasing mucinous component (for unit increase of 10 %; multivariate HR 0.99; 95 % CI 0.95–1.04).

As secondary analyses, we examined the relationship between signet-ring cell component and patient survival in strata of sex or tumor location. Increasing signet-ring cell component was associated with higher CRC-specific mortality both in men (for unit increase of 10 %; multivariate HR 1.17; 95 % CI 1.05–1.32) and women (for unit increase of 10 %; multivariate HR 1.15; 95 % CI 1.06–1.25) ($P_{\text{interaction with sex}} = 0.79$). The association of signet-ring cell component with higher CRC-specific mortality appeared to be evident in colon carcinoma (for unit increase of 10 %; multivariate HR 1.17; 95 % CI 1.09–1.25), but

not in rectal carcinoma (for unit increase of 10 %; multivariate HR 1.16; 95 % CI 0.97–1.38) ($P_{\text{interaction}}$ with tumor location = 0.94), although statistical power was limited in this subgroup analysis.

Furthermore, we examined the interaction between signet-ring cell or mucinous component and each of variables, including disease stage, sex (for mucinous component), age, family history of CRC, tumor location (for mucinous component), tumor differentiation, MSI, CIMP, MLH1 promoter hypermethylation, *KRAS*, *BRAF*, *PIK3CA*, and LINE-1 methylation. We did not observe statistically significant interaction after correcting for multiple hypothesis testing [all $P_{\text{interaction}} > 0.03$; given multiple hypothesis testing, a statistical significance level was adjusted to $P_{\text{interaction}} = 0.004 (=0.05/13)$].

MSI Status and Mortality, Stratified by Proportion of Signet-Ring Cell/Mucinous Component

Considering recent studies of prognostic association between MSI status and signet-ring cell/mucinous carcinoma, we additionally examined the prognostic association of MSI status in strata of the proportion of signet-ring cell component (Supplementary Table 2) and mucinous component (Supplementary Table 3).^{14,39–43} CRC-specific mortality for signet-ring cell carcinoma (CRC with signet-ring cell component >50 %) did not differ by MSI status, although statistical power was limited in this subgroup analysis. With respect to mucinous carcinoma (CRC with mucinous component >50 %), mucinous carcinoma with MSI-high was associated with lower CRC-specific mortality than mucinous carcinoma with MSS in univariate and multivariate analysis (multivariate HR 0.27; 95 % CI 0.10–0.72, Supplementary Table 3). Lower multivariate HR estimate of 0.27 than stage-stratified HR estimate of 0.41 was essentially due to adjusting for *BRAF* mutation; when we simply adjusted for *BRAF* mutation in a stage-stratified model, adjusted HR for CRC-specific mortality was 0.30 (0.11–0.79). No other major confounder was identified.

The prognostic associations of each molecular feature (MSI, CIMP, *KRAS*, *BRAF*, *PIK3CA*, and LINE-1 methylation) are provided in Supplementary Table 4.

DISCUSSION

The unique resource of a molecular pathological epidemiology database, containing a large number of CRCs with detailed molecular analyses in two cohort studies, enabled us to examine the prognostic association of signet-ring cell and mucinous components in CRC patients, independent of various tumor molecular and other clinicopathologic features. We found that even 50 % or less of signet-ring cell component was associated with higher mortality, independent of other clinicopathologic and a number of tumor molecular features. On the other hand, we observed no association between mucinous component and CRC patient mortality. Our results provide useful information on signet-ring cell/mucinous histology in clinical and pathological practice of CRC.

Examining histopathological and tumor molecular features has become increasingly important in CRC and other cancers.^{44–51} Signet-ring cell carcinoma and mucinous carcinoma are subtypes of adenocarcinoma with prominent mucin secretion and share

molecular features, including MSI-high, CIMP-high, higher LINE-1 methylation level, and frequent *BRAF* mutation.^{8,12–16} MSI, CIMP, LINE-1 methylation, and *BRAF* mutation status have been independently associated with prognosis of CRC patients.^{17–19} Although these factors can confound each other in survival analysis, few studies comprehensively examined them beyond MSI status to assess the independent prognostic significance of signet-ring cell or mucinous component (or carcinoma).^{14,39–43,52} Our current study is, therefore, of particular importance in that we examined the prognostic association of signet-ring cell and mucinous components, independent of tumor molecular features. Furthermore, by using the proportion of each component, which is not routinely recorded in pathological reports, we could evaluate the prognostic significance of a minor component.

Similar to mucinous carcinoma, signet-ring cell carcinoma is associated with MSI-high and molecular features common to other MSI-high CRCs.^{16,19,53} Although MSI-high is a well-established prognostic biomarker for better survival, signet-ring cell carcinoma is associated with shorter survival in CRC patients. Due to the rarity, however, few studies have investigated its molecular etiology.^{12,54–56} Further studies are warranted to elucidate molecular mechanisms accounting for the aggressive behavior.

Importantly, we found that even 50 % or less of signet-ring cell component is associated with higher mortality in CRC patients. The prognostic significance of a minor signet-ring cell component warrants a recommendation that pathologists try to identify and report any minor signet-ring cell component. Nevertheless, we should keep in mind that the low number of signet-ring cell carcinoma ($N = 20$) contributed to the association between signet-ring cell component and higher mortality.

Several limitations of our study deserve comment. First, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use substantially differed according to signet-ring cell or mucinous histology, given evidence suggesting no difference in the efficacy of chemotherapy depending on these histologies.^{57–59} In addition, our multivariate survival analyses were finely adjusted for disease stage, on which treatment decisions are mainly based. Second, data on recurrence time and patterns were unavailable, beyond cause of mortality. Nonetheless, given a median follow-up of over 12 years for censored cases, CRC-specific mortality might be a reasonable surrogate of CRC-specific outcomes. Third, we excluded cases without available tumor tissue, which might cause bias. Nonetheless, a previous study has shown that there are no substantial demographic or clinical differences between cases with available tumor tissue and cases without available tumor tissue.²⁸

In conclusion, our study demonstrated that even 50 % or less of signet-ring cell component is associated with higher mortality in CRC patients, independent of other clinico-pathological and tumor molecular characteristics. Further studies are required to confirm our results as well as to elucidate molecular mechanisms by which signet-ring cell component affects behavior of CRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Kaplan-Meier curves for CRC-specific survival (a) and overall survival (b) according to the proportion of signet-ring cell component (0, 1–50, >50 %). Table shows the number of patients who remained alive, and at risk of death, at each time point after the diagnosis of CRC

FIG. 2.

Kaplan-Meier curves for CRC-specific survival (a) and overall survival (b) according to the proportion of mucinous component (0, 1–50, >50 %). Table shows the number of patients who remained alive, and at risk of death, at each time point after the diagnosis of CRC

TABLE 1
Clinical, pathological, and molecular characteristics according to signet-ring cell component and mucinous component in CRC

Clinical, pathological, or molecular feature	N	Signet-ring cell component			P	Mucinous component			P
		0 %	1-50 %	>50 %		0 %	1-50 %	>50 %	
All cases	1,336	1,190	126	20		825	371	140	
Sex					0.92				1.00
Male (HPFS)	585 (44 %)	523 (44 %)	52 (41 %)	10 (50 %)		358 (43 %)	169 (46 %)	58 (41 %)	
Female (NHS)	751 (56 %)	667 (56 %)	74 (59 %)	10 (50 %)		467 (57 %)	202 (54 %)	82 (59 %)	
Age (year) mean $\pm$ SD	68.3 $\pm$ 8.8	68.1 $\pm$ 8.9	70.6 $\pm$ 8.2	68.2 $\pm$ 7.4	0.011	67.7 $\pm$ 8.9	69.4 $\pm$ 9.0	69.1 $\pm$ 7.8	0.0046
Year of diagnosis					0.0026				0.0012
Before 1998	696 (52 %)	641 (54 %)	44 (35 %)	11 (55 %)		460 (56 %)	173 (47 %)	63 (45 %)	
1998-2008	640 (48 %)	549 (46 %)	82 (65 %)	9 (45 %)		365 (44 %)	198 (53 %)	77 (55 %)	
Family history of CRC in first-degree relative					0.65				0.79
No	1,029 (77 %)	921 (78 %)	91 (73 %)	17 (85 %)		636 (77 %)	287 (77 %)	106 (76 %)	
Yes	302 (23 %)	265 (22 %)	34 (27 %)	3 (15 %)		185 (23 %)	84 (23 %)	33 (24 %)	
Tumor location					<0.0001				<0.0001
Cecum	234 (18 %)	205 (17 %)	27 (22 %)	2 (11 %)		115 (14 %)	92 (25 %)	27 (20 %)	
Ascending to transverse colon	400 (30 %)	320 (27 %)	68 (56 %)	12 (63 %)		193 (24 %)	141 (38 %)	66 (49 %)	
Splenic flexure to sigmoid colon	400 (30 %)	381 (32 %)	17 (14 %)	2 (11 %)		287 (35 %)	87 (24 %)	26 (19 %)	
Rectum	289 (22 %)	276 (23 %)	10 (8.2 %)	3 (16 %)		225 (27 %)	49 (13 %)	15 (11 %)	
Tumor differentiation					<0.0001				0.0021
Well to moderate	1,191 (90 %)	1,096 (93 %)	95 (75 %)	0 (0 %)		745 (92 %)	331 (89 %)	115 (83 %)	
Poor	133 (10 %)	82 (7.0 %)	31 (25 %)	20 (100 %)		69 (8.5 %)	40 (11 %)	24 (17 %)	
Disease stage					0.047				0.51
I	318 (24 %)	294 (25 %)	22 (17 %)	2 (10 %)		208 (25 %)	85 (23 %)	25 (18 %)	
II	385 (29 %)	343 (29 %)	38 (30 %)	4 (20 %)		208 (25 %)	119 (32 %)	58 (41 %)	
III	341 (26 %)	300 (25 %)	32 (25 %)	9 (45 %)		225 (27 %)	92 (25 %)	24 (17 %)	
IV	170 (13 %)	140 (12 %)	28 (22 %)	2 (10 %)		101 (12 %)	45 (12 %)	24 (17 %)	
Unknown	122 (9.1 %)	113 (9.5 %)	6 (4.8 %)	3 (15 %)		83 (10 %)	30 (8.1 %)	9 (6.4 %)	
MSI status					<0.0001				<0.0001
MSI-low/MSS	1,015 (84 %)	934 (87 %)	69 (61 %)	12 (71 %)		670 (91 %)	264 (78 %)	81 (63 %)	

Clinical, pathological, or molecular feature	N	Signet-ring cell component			P	Mucinous component			P
		0 %	1–50 %	>50 %		0 %	1–50 %	>50 %	
MSI-high	190 (16 %)	141 (13 %)	44 (39 %)	5 (29 %)		66 (9.0 %)	76 (22 %)	48 (37 %)	
CIMP status					<0.0001				<0.0001
CIMP-low/negative	962 (83 %)	891 (86 %)	59 (56 %)	12 (71 %)		640 (90 %)	240 (74 %)	82 (66 %)	
CIMP-high	200 (17 %)	148 (14 %)	47 (44 %)	5 (29 %)		73 (10 %)	84 (26 %)	43 (34 %)	
<i>MLH1</i> promoter hypermethylation					<0.0001				<0.0001
No	1,003 (86 %)	922 (89 %)	69 (65 %)	12 (71 %)		655 (92 %)	260 (80 %)	88 (70 %)	
Yes	159 (14 %)	117 (11 %)	37 (35 %)	5 (29 %)		58 (8.1 %)	64 (20 %)	37 (30 %)	
<i>KRAS</i> mutation					0.38				0.042
No	780 (64 %)	696 (64 %)	68 (60 %)	16 (94 %)		508 (68 %)	183 (54 %)	89 (68 %)	
Yes	440 (36 %)	393 (36 %)	46 (40 %)	1 (5.9 %)		241 (32 %)	158 (46 %)	41 (32 %)	
<i>BRAF</i> mutation					<0.0001				<0.0001
No	1,040 (85 %)	952 (88 %)	77 (67 %)	11 (65 %)		679 (91 %)	282 (80 %)	89 (68 %)	
Yes	179 (15 %)	135 (12 %)	38 (33 %)	6 (35 %)		68 (9.1 %)	70 (20 %)	41 (32 %)	
<i>PIK3CA</i> mutation					0.43				0.032
No	943 (83 %)	835 (83 %)	93 (84 %)	15 (94 %)		604 (87 %)	234 (75 %)	105 (86 %)	
Yes	187 (17 %)	168 (17 %)	18 (16 %)	1 (6.3 %)		94 (13 %)	76 (25 %)	17 (14 %)	
LINE-1 methylation level, %, mean $\pm$ SD	62.6 $\pm$ 9.5	62.1 $\pm$ 9.3	66.6 $\pm$ 9.9	68.2 $\pm$ 8.3	<0.0001	61.4 $\pm$ 9.2	64.1 $\pm$ 9.6	65.4 $\pm$ 9.3	<0.0001

Percentages indicate the proportion of cases with a specific clinical, pathological, or molecular feature among each subgroup with a specific category of signet-ring cell component or mucinous component. The *P* value for statistical significance was adjusted for multiple hypothesis testing to $P = 0.05/28 = 0.0018$. Thus, a *P* value between 0.05 and 0.0018 should be regarded as of borderline statistical significance

CRC colorectal carcinoma, *CIMP* CpG island methylator phenotype, *HPFS* Health Professionals Follow-up Study, *LINE-1* long interspersed nucleotide element-1, *MSI* microsatellite instability, *MSS* microsatellite stable, *NHS* Nurses' Health Study

TABLE 2

Signet-ring cell component/mucinous component in CRC and patient mortality

Characteristic	CRC-specific mortality					Overall mortality				
	No. of cases	No. of events	Univariate HR (95 % CI)	Stage-stratified HR (95 % CI)	Multivariate HR (95 % CI)	No. of events	Univariate HR (95 % CI)	Stage-stratified HR (95 % CI)	Multivariate HR (95 % CI)	
Signet-ring cell component										
0%	1,190	324	1 (referent)	1 (referent)	1 (referent)	615	1 (referent)	1 (referent)	1 (referent)	
1–50 %	126	47	1.61 (1.18–2.19)	1.29 (0.94–1.77)	1.40 (1.02–1.93)	67	1.39 (1.08–1.79)	1.20 (0.93–1.55)	1.18 (0.90–1.54)	
>50 %	20	13	3.58 (2.05–6.27)	4.11 (2.30–7.32)	4.53 (2.53–8.12)	16	2.58 (1.56–4.25)	2.65 (1.60–4.41)	2.62 (1.56–4.40)	
<i>P</i> _{trend}			<0.0001	<0.0001	<0.0001		<0.0001	0.0012	0.0026	
Mucinous component										
0%	825	237	1 (referent)	1 (referent)	1 (referent)	432	1 (referent)	1 (referent)	1 (referent)	
1–50 %	371	102	1.01 (0.80–1.27)	1.13 (0.90–1.43)	1.04 (0.81–1.33)	189	1.06 (0.89–1.26)	1.12 (0.95–1.34)	1.08 (0.90–1.29)	
>50 %	140	45	1.19 (0.86–1.64)	1.13 (0.82–1.57)	0.82 (0.54–1.23)	77	1.20 (0.94–1.54)	1.14 (0.89–1.46)	0.91 (0.66–1.24)	
<i>P</i> _{trend}			0.39	0.29	0.57		0.14	0.15	1.00	

The multivariate Cox regression models were stage stratified. In addition to signet-ring cell component variable (0, 1–50, >50 %; ordinal), covariates initially included, sex, age at diagnosis, year of diagnosis, family history of colorectal carcinoma, tumor location, tumor differentiation (only for analyses of mucinous component), microsatellite instability, CpG island methylator phenotype, LINE-1 methylation, and *KRAS*, *BRAF*, and *PIK3CA* mutations. P_{trend} values (two-sided) were calculated by using signet-ring cell component/mucinous component as an ordinal categorical variable

CRC colorectal carcinoma, *CI* confidence interval, *HR* hazard ratio