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Unique patterns of CpG island methylation in inflammatory bowel disease-associated colorectal cancers

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Abstract

Objective—CpG island (CGI) hypermethylation at discrete loci is a prevalent cancer-promoting abnormality in sporadic colorectal carcinomas (S-CRCs). We investigated genome-wide CGI methylation in inflammatory bowel disease (IBD)-associated CRCs (IBD-CRCs).

Design—Methylation microarray analyses were conducted on 7 IBD-CRCs, 17 S-CRCs, and 8 normal control colonic tissues from patients without CRC or IBD. CGI methylator phenotype (CIMP), a surrogate marker for widespread cancer-specific CGI hypermethylation, was examined in 30 IBD-CRCs and 43 S-CRCs.

Results—The genome-wide CGI methylation pattern of IBD-CRCs was CIMP status-dependent. Based on methylation array data profiling of all autosomal loci, CIMP⁺ IBD-CRCs grouped together with S-CRCs, while CIMP[−] IBD-CRCs grouped together with control tissues. CIMP[−] IBD-CRCs demonstrated less methylation than did age-matched CIMP[−] S-CRCs at all autosomal CGIs (z-score −0.17 vs. 0.09, $p=3\times10^{-3}$) and CRC-associated hypermethylation target CGIs (z-score −0.43 vs. 0.68, $p=1\times10^{-4}$). Age-associated hypermethylation target CGIs were significantly overrepresented in CGIs that were hypermethylated in S-CRCs ($p=1\times10^{-192}$), but not in CGIs that were hypermethylated in IBD-CRCs ($p=0.11$). In contrast, *KRAS* mutation prevalence were similar between IBD-CRCs and S-CRCs. Notably, CIMP⁺ prevalence was significantly higher in older than in younger IBD-CRC cases (4.2% vs. 50.0%, $p=0.02$), but not in S-CRC cases (16.7% vs. 9.7%, $p=0.92$).

Conclusions—Cancer-specific CGI hypermethylation and age-associated CGI hypermethylation are diminished in IBD-CRCs relative to S-CRCs, while *KRAS* mutation rate is comparable between these cancers. CGI hypermethylation appears to play only a minor role in IBD-associated

carcinogenesis. We speculate that aging, rather than inflammation *per se*, promotes CIMP⁺ CRCs in IBD patients.

Keywords

ulcerative colitis; Crohn's disease; inflammatory bowel disease; colorectal cancer; DNA methylation microarray

INTRODUCTION

Inflammatory bowel disease (IBD) is linked to an elevated risk of developing colorectal cancer (CRC) (1). Severe and longstanding inflammation is considered to underlie IBD-associated CRCs (IBD-CRCs), while aging has been implicated in the genesis of sporadic CRC (S-CRC) (1). Previous studies have elicited contrasts between IBD-CRCs and S-CRCs regarding the frequency and timing of molecular genetic abnormalities (reviewed in refs. (2, 3)), further supporting the existence of distinct cancer-promoting pathways in IBD-CRCs vs. S-CRCs.

CpG island (CGIs) hypermethylation is a frequent epigenetic abnormality among S-CRCs and is linked to aberrant silencing of multiple tumor suppressor genes (4). Polyclonal CGI hypermethylation has been considered to occur in response to endogenous/exogenous stimuli and to serve as a surrogate for genetic mutations in early-stage carcinogenesis (5). CGI hypermethylation occurs in non-neoplastic aging cells, as well as in morphologically normal mucosae adjacent to CRCs, and this hypermethylation process progresses even after neoplastic transformation occurs (6, 7). *MLH1*, *p16*, and *APC*, key tumor suppressor genes in the colon, exhibit this escalating pattern of aberrant hypermethylation (7, 8). Thus, CGI hypermethylation is widely regarded as a key mechanism underlying sporadic neoplastic transformation in the colon (9).

Some CGIs demonstrate tightly concordant cancer-specific hypermethylation (CGI methylator phenotype, or CIMP) in a subset of S-CRCs. CIMP⁺ S-CRCs display unique phenotypic characteristics: *i.e.*, microsatellite instability, frequent mutations of the proto-oncogene *BRAF*, rare chromosomal instability, proximal anatomic location, late age of onset, poor histological differentiation, low cancer-specific mortality, and serrated adenoma origin (10–15). These observations have prompted the proposition of CIMP as the hallmark of an epigenetic silencing-driven carcinogenic pathway, in contradistinction to the aneuploidy/mutation-driven chromosomal instability pathway (16, 17).

The relevance of CGI hypermethylation to the presumed inflammation-driven genesis of IBD-CRCs has been controversial. IBD-CRCs often exhibit CGI hypermethylation of *SOCS3*, a gene whose inactivation causes hyperactivity of the inflammation-related IL6-STAT3 signaling cascade and consequent tumor initiation (18, 19). IL-6 has also been reported to upregulate DNA methyltransferase via suppression of DNA methyltransferase-targeting microRNAs (20, 21). Moreover, non-neoplastic IBD colonocytes show accelerated age-associated CGI hypermethylation, suggesting the further promotion of CGI hypermethylation by inflammation (22, 23). Nevertheless, the prevalence of cancer-specific CGI hypermethylation in IBD-CRCs is variable when studied at selected single-target loci (22, 24–28). Therefore, in the current study, we assessed the relevance of CGI hypermethylation to IBD-carcinogenesis by performing microarray-based genome-wide analyses of CGI methylation and by analyzing phenotype-CIMP status associations.

MATERIALS AND METHODS

Patients

IBD-associated colorectal cancer (IBD-CRC) and sporadic CRC (S-CRC) tissues were obtained from tissue repositories at the Mount Sinai Hospital and the Johns Hopkins Hospital, respectively. Tissues were macroscopically dissected to enrich tumor cells prior to cryostorage. The presence of histologically active colitis in adjacent mucosae was verified for all IBD-CRC samples, based on criteria established by one of the authors (N.H.) at the Mount Sinai Hospital (29). Control colonic mucosal biopsy specimens were obtained at screening colonoscopies performed on patients who had no history of IBD, colonic neoplasia, celiac disease, or other colonoscopic abnormalities at the Johns Hopkins Hospital. Tissues were acquired according to protocols approved by institutional review committees at the Johns Hopkins University, Baltimore, MD, and the Mount Sinai Medical Center, New York, NY. Written consent was obtained from each participating patient. Genomic DNA was extracted from snap-frozen tissues using a DNeasy minicolumn kit (Qiagen, Valencia, CA). Enrollment of a banked specimen in the study was based on quantity and integrity of the extracted DNA as well as demographic data. IBD-CRCs that developed within 2 years after onset of IBD, in lesions lacking clinical or histological evidence of current or previous persistent inflammation, or in the setting of indeterminate colitis were excluded from the study. IBD-CRCs and SCRCs with a previous history of CRC, chemotherapy, or radiotherapy prior to tissue sampling were also excluded. Demographic data for cases studied in microarray experiments and CIMP status assessments are detailed in Tables 1a and 1b, respectively.

Methylated CpG island Amplification coupled with Microarray (MCAM) analysis

Selective enrichment of methylated DNA in each sample DNA was conducted based on serial digestion with a set of isoschizomers, methylation-sensitive *SmaI* and methylation-insensitive *XmaI*, followed by *XmaI*-digested fragment-specific linker PCR, as described previously (30). 244K Human CpG Island microarrays (Agilent Technologies, Santa Clara, CA) were used as an array platform, and fully methylated DNA was used as the control specimen, as described previously (31). LOESS normalization was carried out, and normalized log₂ intensity ratio to a fully methylated control DNA at each locus was used as the value representing locus methylation level. Significant differential methylation was defined as mean normalized log₂ intensity ratio difference greater than 1 at t-test p-level less than 0.01. Further details are described in Supplementary Materials. We previously verified low experimental batch variance of this MCAM protocol in our hands and high correlation between MCAM-based- and real-time quantitative methylation-specific PCR-based methylation measurements (31).

Diagnosis of CpG island methylator phenotype (CIMP) status and KRAS mutation

CIMP status of each tumor was determined based on the methylation status of five loci (*CACNA1G*, *IGF2*, *RUNX3*, *SOC31*, and *NEUROG*) that were measured by real-time quantitative methylation-specific PCR (qMSP) assays, as previously described (32). CRCs demonstrating methylation at more than 60% of informative loci were classified as CIMP⁺ cancers. Mutation at *KRAS* cods 12 and 13 was evaluated by pyrosequencing analysis as described previously (33).

Statistical analyses

Throughout this paper, mean $\pm$ standard deviation (SD) were used as the data representing a group of specimens or genes, unless otherwise stated. The unpaired two-tailed Student's t-test was applied to comparisons of two groups based on continuous variables, while Chi-

squared or two-tailed Fisher's exact tests were applied to categorical data-based comparisons of multiple or two groups, respectively. Correlation coefficient between age and locus methylation did not follow normal distribution and was assessed by Mann-Whitney's U-test. P-values less than 0.05 were considered statistically significant, unless otherwise stated. Nonsignificant trend was noted at p-value less than 0.1. Cluster analysis was conducted using Cluster 3.0 and TreeView (Michael Eisen, Stanford University).

RESULTS

CpG island hypermethylation is significantly rarer in inflammatory bowel disease-associated colorectal cancers (IBD-CRCs) than in sporadic colorectal cancers (S-CRCs)

Locus-specific CGI methylation level was analyzed in a genome-wide fashion utilizing MCAM, which enabled us to evaluate methylation of 34,396 DNA regions corresponding to 14,213 CGIs (*i.e.*, 50.4% of all CGIs in the genome; *vide supra*). The majority of these *SmaI-XmaI* fragments were overlapped with either gene promoter regions (63%) or transcribed regions (17%). We analyzed 7 IBD-CRCs (one CIMP⁺ and 6 CIMP⁻ CRCs), 17 S-CRCs (5 CIMP⁺ and 12 CIMP⁻ CRCs), and 8 control colonic mucosae from patients with no history of IBD or colonic neoplasia.

Cluster analysis of the array data set comprising all 34,341 loci was performed to evaluate similarities and differences in CGI methylation patterns associated with neoplastic status, presence of IBD, and CIMP status (Figure 1). As expected, cluster analysis distinguished S-CRCs from controls, regardless of their CIMP status. Cluster analysis also revealed a large number of loci that were hypermethylated in S-CRCs relative to controls. In contrast, among IBD-CRCs, only the CIMP⁺ case clustered together with S-CRCs. All CIMP⁻ IBD-CRCs clustered with controls and generally lacked hypermethylation at loci where the majority of CIMP⁻ S-CRCs demonstrated hypermethylation. There were no clusters of loci that were uniquely hyper- or hypomethylated in IBD-CRCs.

We then used the array data to calculate the mean z-score for all autosomal loci for each specimen. The results of the z-score analysis were consistent to the observation made in the cluster analysis. Significantly greater mean z-scores relative to the 8 controls (-0.21 ± 0.13) were observed in both the 5 CIMP⁺ S-CRCs (0.25 ± 0.14 , $p=2 \times 10^{-4}$) and the 12 CIMP⁻ S-CRCs (0.10 ± 0.09 , $p=3 \times 10^{-6}$). Similarly to the CIMP⁺ S-CRCs, the CIMP⁺ IBD-CRC exhibited an elevated mean z-score (0.45) that was greater than the mean+3SD z-score of control specimens (*i.e.*, 0.18). However, the 5 CIMP⁻ IBD-CRCs showed no significant elevation in mean z-score (-0.17 ± 0.10) relative to the 8 controls (-0.21 ± 0.13 , $p=0.44$).

Analysis of an age- and CIMP-status matched tumor subset confirms significantly rarer CpG island hypermethylation in IBD-CRCs than in S-CRCs

CRCs generally develop at younger ages in IBD than in the sporadic setting, and aging is known to promote CGI hypermethylation (1, 9). To eliminate the impact of age differences, we compared an age-matched CIMP⁻ subset consisting of 4 S-CRCs (55.2 ± 5.2 years), 4 IBD-CRCs (53.0 ± 13.1 years; $p=0.71$; Table 3). Significant overall z-score elevation relative to controls (-0.21 ± 0.13) remained present in S-CRCs (0.04 ± 0.06 , $p=4 \times 10^{-3}$) and remained absent in IBD-CRCs (-0.17 ± 0.08 , $p=0.55$). The mean z-score of the 6,414 loci that showed S-CRC-associated hypermethylation was significantly smaller in IBD-CRCs than in S-CRCs (-0.43 ± 0.25 vs. 0.68 ± 0.04 , respectively, $p=1 \times 10^{-4}$; Table 2).

Age-associated CpG island hypermethylation in IBD-CRCs is less severe than in S-CRCs

Acceleration of age-associated hypermethylation in IBD is well-documented and has been a candidate cancer promoting factor (22, 34). Therefore, we analyzed the mean z-score for the 834 age-associated hypermethylation target loci that were identified by a genome-wide study (35). There was an insignificant trend toward decrease in mean z-score in CIMP⁻ IBD-CRCs relative to in CIMP⁻ S-CRCs (Table 2). We also evaluated the enrichment of age-associated hypermethylation target loci in loci that were hypermethylated in CRCs using the same age-matched CIMP⁻ CRC cohort. We used correlation coefficient *R* between age and locus methylation level in non-IBD non-CRC controls as the indicator of age-associated hypermethylation. Relative to baseline (i.e., all autosomal loci, median *R*=0.27), loci showing strong age-associated hypermethylation were significantly enriched in loci that were hypermethylated in S-CRC (median *R*=0.62, $p=1\times10^{-192}$) but not in loci that were hypermethylated in IBD-CRCs (median *R*=0.26, $p=0.11$; Figure 2).

CpG island methylator phenotype is associated with older age in IBD-CRCs

Because a CIMP status-dependent discrepancy in global CGI methylation profile was observed in IBD-CRCs, we assessed the association between CIMP status and demographic data in a larger CRC cohort (30 IBD-CRCs and 43 S-CRCs). No significant difference in *KRAS* mutation rate was observed between IBD-CRCs and S-CRCs (9 of 29, 31.0% vs. 11 of 43, 25.6%, $p=0.61$) in this cohort, consistent to a recent report (Table 1b) (36). No significant difference was observed between IBD-CRCs and S-CRCs in CIMP⁺ prevalence (4 of 30, 13.3% vs. 5 of 43, 11.6%, $p=1.0$; Table 3). However, CIMP⁺-CRC incidence was significantly higher in the old (i.e., 60 years and older) than in the young (i.e., younger than 60 years) IBD-CRC cases (1 of 24, 4.0%, vs. 3 of 6, 50.0%; $p=0.02$, Table 3). In contrast, CIMP⁺-CRC incidence did not differ between old and young S-CRCs (2 of 12, 16.7%, vs. 3 of 31, 9.7%; $p=0.92$). No significant association was observed between CIMP status and gender, tumor stage, IBD disease subtype, or *KRAS* mutation status.

DISCUSSION

Increased abnormal CGI hypermethylation-mediated tumor suppressor gene silencing has been proposed as a link between IBD-associated inflammation and carcinogenesis (reviewed in ref. (1)). Nevertheless, the relevance of CGI hypermethylation-mediated gene silencing in IBD-associated carcinogenesis remains controversial, considering the highly variable gene methylation prevalence reported in IBD-CRCs (22, 24–28). Our methylation microarray experiments confirmed infrequent CRC-associated CGI hypermethylation in CIMP⁻ IBD-CRCs relative to age-matched sporadic counterparts. A previous study of selected CRC-specific hypermethylation targets reported the same phenomenon (28). However, this previous study did not exclude the possibility that rarer cancer-specific hypermethylation in IBD-CRCs was merely due to: a) fewer CIMP⁺ CRCs in the IBD-CRC cohort (17%) than in the S-CRC cohort (38%) and b) studying CRC-specific hypermethylation targets also known to be markers that differentiate CIMP⁺ CRCs from CIMP⁻ CRCs. Our results were not confounded by these potential biases, but still conclusively demonstrated a lack of CRC-associated hypermethylation in IBD-CRCs relative to S-CRCs.

Accelerated aging-associated hypermethylation has been well-documented in the precancerous colonic mucosae of IBD patients and is suspected of contributing to IBD-associated carcinogenesis (22, 26, 34, 37). However, contrary to this widely held hypothesis, our current findings established that age-associated hypermethylation target loci lacked hypermethylation in CIMP⁻ IBD-CRCs. This finding is consistent with a previous study of selected individual age-associated hypermethylation targets (28). It also suggests the possibility that colonocytes manifesting with accelerated age-associated hypermethylation in

IBD do not undergo selection during carcinogenic progression. This low rate of age-associated hypermethylation in IBD-CRCs parallels the overall rarity of cancer-specific hypermethylation in these tumors. Taken together, these data imply that inflammation-driven CGI hypermethylation plays only a minor role in IBD-associated colorectal carcinogenesis.

In contrast to their relative lack of CGI hypermethylation, we observed that IBD-CRCs carried similarly frequent *KRAS* mutation to did their S-CRC counterparts, which is consistent with a recent report (36). IBD-associated inflammation promotes cellular turnover and oxidative stress. Aggravated genomic damage (*e.g.*, telomere shortening, chromosomal instability, *TP53* mutation) induced by these factors develops in non-neoplastic colonic mucosae of patients suffering from longstanding and severe IBD (23, 38, 39). Thus, genetic (rather than epigenetic) aberrations seem to play a more dominant role in IBD-associated neoplastic transformation, perhaps eliminating the biological requirement for further methylation-mediated gene dysregulation.

The CIMP⁺ phenotype reflects a distinctive aging-associated carcinogenic pathway in sporadic CRCs, which can be detected as early as at the adenoma stage (12, 40). Considering the S-CRC-like widespread cancer-specific hypermethylation and late onset age of CIMP⁺ IBD-CRCs, it is tempting to speculate that aging, a principal driving force behind sporadic colon carcinogenesis, plays a more important role than does inflammation in the genesis of CIMP⁺ IBD-CRCs. If the CIMP⁺ phenotype corresponds to inflammation-independent carcinogenesis, then identifying CIMP⁺ adenomas may become relevant to managing IBD patients, since conservative polypectomy is recommended for sporadic adenomas occurring in the setting of IBD (41). Unfortunately, we were unable to determine whether CIMP⁺ was an age-dependent, rather than IBD duration-dependent, phenomenon, because many cases in the current cohort lacked disease duration information. Thus, further investigations of larger IBD-neoplasia cohorts will be helpful in further clarifying this issue.

In summary, the current genome-wide methylation analysis reveals that CGI hypermethylation in IBD-CRCs is rare relative to S-CRCs, while genetic aberrations *per se* are comparable between these two colorectal cancer subtypes. A small IBD-CRC subset, CIMP⁺ IBD-CRC, resembles S-CRC more than CIMP⁻ IBD-CRC in its late age of onset and widespread cancer-specific CGI hypermethylation. These findings support the notion that accelerated genesis of point mutations supersedes CGI hypermethylation in IBD-associated carcinogenesis (28, 42).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The genome-wide CpG island methylation profile of IBD-CRCs resembles that of non-IBD non-cancer controls more than that of S-CRCs

This Figure illustrates clustering of 7 IBD-CRCs, 17 S-CRCs, and 8 controls according to the similarity of their genome-wide CpG island methylation profiles. Methylation profiling was performed by k-means clustering of MCAM data for all 34,396 loci. Each small bar shows the methylation level of a specimen at a locus, and data are aligned according to specimen (horizontal alignment) and locus (vertical alignment). The identity of each specimen is indicated by the color of a *rectangle* at the top (*orange*, IBD-CRC; *blue*, S-CRC; *gray*, control). CIMP⁺ CRCs are indicated by *asterisks*. Methylation status-based cluster analysis clearly separated S-CRCs from controls, with multiple loci demonstrating hypermethylation in S-CRCs (indicated by a *black bar*). Hypermethylation of a subset of loci was unique to CIMP⁺ CRCs (indicated by a *brown bar*). In contrast, IBD-CRCs lacked the widespread hypermethylation observed in S-CRCs; IBD-CRCs clustered with controls, with the exception of one CIMP⁺ IBD-CRC. This CIMP⁺ IBD-CRC clustered with, and shared the characteristic widespread hypermethylation of, S-CRCs.

Figure 2. Age-associated hypermethylation targets are over-represented in S-CRC-associated hypermethylation targets but not in IBD-CRC-associated hypermethylation targets
 These box plots show the degree of age-associated hypermethylation (i.e., correlation coefficient R between age and methylation level in non-IBD non-CRC controls) for loci that falls into the following four categories: *all autosomal* loci analyzed by the array (i.e., baseline, $n=34,341$), loci showing significant hypermethylation in *IBD-CRCs* ($n=2,418$), loci showing significant hypermethylation in *S-CRC* ($n=6,464$), and previously reported age-associated hypermethylation targets (i.e., positive control, $n=834$). *Dots*, *boxes*, and *whiskers* indicate median, 25–75 percentile range, and 10–90 percentile range, respectively. *Asterisks* indicate statistically significant difference from baseline.

Table 1a

Demographic data for specimens used in the MCAM analysis

Tissues type	IBD-CRC	S-CRC	non-IBD non-CRC control
Total number	7	17	8
Age			
Mean $\pm$ SD (Min.-Max.)	<i>a</i> . <i>b</i> 49.0 $\pm$ 14.6 (29-72)	63.2 $\pm$ 10.8 (36-80)	63.3 $\pm$ 8.9 (55-77)
Gender			
F	2 (28.6%)	5 (29.4%)	0 (0%)
M	5 (71.4%)	12 (70.6%)	8 (100%)
Site			
R	<i>c</i> ₁ (14.3%)	10 (58.8%)	4 (50.0%)
L	6 (85.7%)	7 (41.2%)	4 (50.0%)
Stage			
AB	4 (57.1%)	8 (47.1%)	NA
CD	3 (42.9%)	9 (52.9%)	NA
Histological grade			
well-moderate	<i>b</i> ₀ (0%)	9 (60.0%)	NA
poor	7 (100%)	6 (40.0%)	NA
unknown	0	2	NA
CIMP			
CIMP ⁺	1 (14.3%)	5 (29.4%)	NA
CIMP ⁻	6 (85.7%)	12 (70.6%)	NA
IBD subtype			
UC	4 (57.1%)	NA	NA
CD	3 (42.9%)	NA	NA

^a, p<0.05 in comparison to control;

^b, $p < 0.05$ in comparison to S-CRC;

^c, $p < 0.10$ in comparison to S-CRC; SD, standard deviation; Min., minimum; max., maximum; UC, ulcerative colitis; CD, Crohn's disease; NA, not applicable. All UC cases had pancolitis, and all CD cases had ileocolitis.

Table 1b

Demographic data for specimens used in CIMP status analysis.

Tumor type	IBD-CRC	SCRC	P-value
Total number	30	43	
Age			p=3E-6
Mean+/-SD (Min.-Max.)	49.8+/-15.5 (27-84)	65.3+/-10.4 (36-81)	
Gender			p=0.02
F	13 (43.3%)	7 (16.3%)	
M	17 (56.7%)	36 (83.7%)	
Site			p=1.00
R	12 (41.4%)	18 (41.9%)	
L	17 (58.6%)	25 (58.1%)	
unknown	1	0	
Stage			p=0.09
AB	19 (67.9%)	19 (43.2%)	
CD	9 (32.1%)	24 (55.8%)	
unknown	2	0	
Histological grade			p=0.03
well-moderate	15 (50.0%)	32 (78.1%)	
poor	15 (50.0%)	9 (21.9%)	
unknown	0	2	
IBD subtype			NA
UC	26 (86.7%)	NA	
CD	4 (13.3%)	NA	
IBD extent			NA
UC-pancolitis	15	NA	
UC-left sided	0	NA	
UC-proctosigmoiditis	0	NA	
UC-unknown	11	NA	
CD-ileitis	2	NA	
CD-ileocolitis	2	NA	
CD-unknown	0	NA	
KRAS mutation			p=0.61
positive	9 (31.0%)	11 (25.6%)	

Tumor type	IBD-CRC	SCRC	P-value
negative	20 (69.0%)	32 (74.4%)	
unknown	1	0	

Table 2

z-score comparison of CIMP[−] IBD-CRCs and age-matched CIMP[−] S-CRCs.

Categories	No. of loci	IBD-CRC mean (SD)	S-CRC mean (SD)	p-value
Case age (years)	NA	53.0 (13.1)	55.8 (5.2)	0.71
z-score: all autosomal loci	33,414	−0.17 (0.08)	0.09 (0.07)	3×10^{-3}
z-score: CRC-associated hypermethylation targets	6,464	−0.43 (0.25)	0.68 (0.04)	1×10^{-4}
z-score: aging-associated hypermethylation targets	834	−0.38 (0.28)	0.15 (0.53)	0.07

The mean value of z-scores corresponding to the indicated locus (or each of the indicated loci) was calculated for each sample. Average and SD (in parenthesis) of these mean z-scores for respective sample groups are displayed.

Table 3

CIMP incidence by clinical and demographical data.

Tumor type	IBD-CRCs	SCRCs	P-value
Total number	4 of 30 (13.3%)	5 of 43 (11.6%)	1.00
Age			IBDN: 0.02; S-CRC: 0.91
<60 years	1 of 24 (4.2%)	2 of 12 (16.7%)	
=>60 years	3 of 6 (50.0%)	3 of 31 (9.7%)	
IBD subtype			IBDN: 1.00; S-CRC: NA
UC	4 of 26 (15.3%)	NA	
CD	0 of 4 (0%)	NA	
Gender			IBD-CRC: 1.00; S-CRC: 1.00
F	2 of 13 (15.4%)	1 of 7 (14.3%)	
M	2 of 17 (11.8%)	4 of 36 (11.1%)	
Stage			IBD-CRC: 0.57; S-CRC: 1.00
AB	2 of 19 (10.5%)	2 of 19 (10.5%)	
CD	2 of 9 (22.2%)	3 of 24 (12.5%)	
Tumor site			IBD-CRC: 0.36; S-CRC: 0.17
R	3 of 12 (25.0%)	4 of 18 (22.2%)	
L	1 of 17 (5.9%)	1 of 25 (4.0%)	
Histological grade			IBD-CRC: 1.00; S-CRC: 0.11
well to moderate	2 of 15 (13.3%)	2 of 32 (6.3%)	
poor	2 of 15 (13.3%)	3 of 9 (33.3%)	
KRAS mutation			IBD-CRC: 0.76; S-CRC: 0.74
Mutant	1 of 9 (11.1%)	1 of 11 (9.1%)	
Wild type	3 of 20 (15.0%)	4 of 32 (12.5%)	