

Molecular Phenotype of Inflammatory Bowel Disease–Associated Neoplasms With Microsatellite Instability

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Background & Aims: Patients with inflammatory bowel disease (IBD) are at increased risk of developing colorectal cancer (CRC). We sought to determine the frequency of high-level microsatellite instability (MSI-H) and the mutational and methylation profile of MSI-H IBD-related neoplasms (IBDNs). **Methods:** A total of 124 IBDNs (81 cancers, 43 dysplasias) from 78 patients were studied for the frequency of MSI-H and hypermethylation of 3 target genes: *MLH1*, *HPP1*, and *RAB-32*. Fifteen MSI-H IBDNs were characterized according to their profile of frameshift mutations in 28 mononucleotide repeats and compared with 46 sporadic MSI-H CRCs. **Results:** Nineteen of 124 IBDNs were MSI-H. The frequency of frameshift mutations in coding mononucleotide repeats was significantly lower in MSI-H IBDNs than in sporadic MSI-H CRCs for *TGFB2* (7 of 14 vs 34 of 43 samples; $P = .047$) and *ACVR2* (3 of 14 vs 25 of 43 samples; $P = .029$). In contrast, *ICA1* was mutated in 3 of 9 MSI-H IBDNs vs 2 of 54 sporadic MSI-H CRCs ($P = .028$). *HPP1* and *RAB32* methylation was independent of MSI status and was observed in 4 of 59 and 0 of 64 nondysplastic mucosae, 20 of 38 and 1 of 25 dysplasias, and 28 of 61 and 20 of 60 carcinomas, respectively. **Conclusions:** The profiles of coding microsatellite mutations (instabilotypes) differ significantly between MSI-H IBDNs and MSI-H sporadic CRCs. Specifically, *TGFB2* and *ACVR2* mutations are significantly rarer in MSI-H IBDNs than in MSI-H sporadic CRCs. Furthermore, *HPP1* methylation occurs early, in 7% of nondysplastic and approximately half of dysplastic mucosae, whereas *RAB32* methylation occurs at the transition to invasive growth, being rarer in dysplasias.

Patients with ulcerative colitis (UC) and Crohn's colitis are at increased risk of developing colorectal cancer (CRC). The duration of inflammatory bowel disease (IBD) is one the most important risk factors impinging on this risk.^{1,2} CRC occurs rarely before a 7-year

duration of colitis. A recent meta-analysis reported a cumulative risk of CRC of 18% after 30 years of disease.³ Another important risk factor for CRC is the extent of the disease, with standardized incidence ratios of 1.7 for proctitis, 2.8 for left-sided colitis, and 14.8 for pancolitis.¹ In addition, a positive family history of CRC and the diagnosis of concomitant primary sclerosing cholangitis have been shown to be associated with an increased risk of CRC in patients with UC.^{4–8} Recently, the histologic degree of inflammation has been proposed as an independent risk factor for CRC in UC.⁹ Patients with longstanding extensive Crohn's colitis are also at an increased risk of CRC.^{2,10} Moreover, the risk of small bowel cancer is significantly increased in patients with Crohn's disease (CD) relative to the general population.² The median age of diagnosis of bowel cancer (CRC and small bowel cancer) in patients with IBD is significantly earlier than in sporadic bowel cancer.^{2,11}

IBD-associated CRCs are different from sporadic carcinoma in several respects. For example, unlike sporadic CRC, which arises from polypoid adenomas, IBD-associated CRC develops from often flat areas of dysplastic mucosa; sporadic adenomas require 10–15 years to evolve in carcinomas, whereas IBD-associated dysplasias generally progress within 3 years. In addition, IBD-associated dysplasias arise within a setting of intense inflammation, whereas sporadic adenomas are not asso-

Abbreviations used in this paper: CRC, colorectal cancer; DALM, dysplasia-associated lesion or mass; HNPCC, hereditary nonpolyposis colorectal cancer; IBDN, inflammatory bowel disease-related neoplasm; IC, indeterminate colitis; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, high-level microsatellite instability; MSI-L, low-level microsatellite instability; MSS, microsatellite stable; NMV, normalized methylation value.

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ciated with inflammation.¹² Although the molecular events that facilitate the progression of adenoma to carcinoma in sporadic CRC have been well investigated,¹³ much remains unclear regarding the molecular events underlying IBD-associated neoplasms (IBDNs). Previous studies have shown important differences between sporadic and IBD-associated neoplasias regarding the frequency and timing of certain genetic alterations. For example, a recent study of gene expression profiles in IBDNs showed significant differences from their sporadic counterparts.¹⁴

A number of studies have focused on markers of chromosomal instability, such as aneuploidy,^{15–19} loss of heterozygosity, genomic losses or gains using comparative genomic hybridization,^{20–29} or mutational or protein expression studies of oncogenes and tumor suppressor genes. Regarding the latter, it was shown that *p53* gene alterations occur early, even in nonneoplastic colonic mucosa of patients with IBD, whereas *APC* alterations, which are common and early in sporadic CRC, were detected rarely and late in IBD-associated carcinogenesis.^{13,26,30–37} Similarly, mutations of *DPC4* and *K-ras* have been shown to be infrequent in IBD-associated cancers,^{36,38–44} whereas they are frequent events in sporadic CRCs.

Studies focusing on microsatellite instability (MSI), a second major form of genomic instability, have been conflicting regarding the frequency of high-level MSI (MSI-H), with reports ranging from <1% to 45% in IBDNs.^{36,44–50} Knowledge regarding the clinical and molecular events underlying IBDNs with MSI-H is limited. Therefore, we determined the frequency of MSI-H in IBDNs, compared the clinical phenotype of MSI-H IBDNs with that of non-IBD MSI-H CRCs, and performed a comprehensive analysis of frameshift mutations in 28 coding mononucleotide repeats in 15 MSI-H IBDNs. Finally, because MSI is closely linked to *MLH1* promoter methylation in sporadic neoplasms, and because *HPP1* and *RAB32* methylation are associated with MSI-H phenotype and *MLH1* methylation in sporadic MSI-H gastric and colon carcinomas, respectively, we performed an analysis of promoter methylation of *MLH1*, *HPP1*, and *RAB32* in IBDNs.

Materials and Methods

Patients

Tissue specimens from 79 CRCs, 43 colorectal dysplasias, and 2 small bowel carcinomas were obtained from 78 patients with IBD undergoing surgery at the Mount Sinai Hospital in New York City. The patient cohort comprised 66 patients with UC, 8 with CD, and 4 with

indeterminate colitis (IC). Specimens were obtained and medical charts were reviewed under a protocol approved by the institutional review boards of the University of Maryland and Baltimore VA Hospitals and the Mount Sinai Hospital in New York City. The clinical characteristics and the profile of frameshift mutations of coding mononucleotide repeats were compared with a previously reported series of 46 non-IBD MSI-H CRCs from 44 patients, including 10 with hereditary nonpolyposis colorectal cancer (HNPCC)-associated CRC and 36 with sporadic CRC.⁵¹ The data for sporadic CRCs analyzed for MSI status and *MLH1* hypermethylation were from a previously reported study.⁵²

Methods

Tissue collection, histologic diagnosis, and DNA extraction. All tissues were harvested postoperatively and stored at -80°C . All tissues were grossly dissected free of normal surrounding tissue, and parallel sections were used for histologic characterization. Classification and grading of dysplasias and carcinomas were carried out according to standardized histologic consensus criteria.⁵³ Dysplastic lesions were classified macroscopically as flat or polypoid (dysplasia-associated lesion or mass [DALM]). Matching normal mucosa was collected in each case. Although laser capture microdissection was not performed, the specimens were determined to contain at least 70% neoplastic cells by H&E staining. Genomic DNA was extracted as described previously.⁴⁰

MSI testing and analysis of frameshift mutations in coding region microsatellites. MSI status and analysis of frameshift mutations in coding mononucleotide repeats were performed as described previously.^{51,54} MSI was analyzed by evaluation of 5 consensus loci (BAT25, BAT26, D2S123, D5S346, and D17S250).⁵⁵ Specimens showing MSI at 2 or more of informative loci were classified as MSI-H, while specimens showing no MSI were classified as microsatellite stable (MSS). Specimens with instability at one marker were classified as low-level MSI (MSI-L). For statistical comparisons, MSS and MSI-L were grouped together as non-MSI-H. Normal tissue for MSI testing was derived from the terminal ileum except in 4 cases of CD, for which it was taken from the uninvolved small bowel ($n = 2$) or colon ($n = 2$). All tissues were not macroscopically inflamed. For coding mononucleotide repeat frameshift mutational analysis, we classified an alteration as tumor-specific when it caused a change of >50% in peak height in the tumor sample compared with the corresponding normal sample. Due to limited DNA amounts, the analysis of coding mononucleotide repeat mutations was limited to 28 genes. Genes were selected based on the frequency of alterations in non-IBD MSI-H CRC and gastric cancers from our previous studies.^{51,54}

Bisulfite treatment and real-time quantitative methylation-specific polymerase chain reaction. DNA was treated with bisulfite to convert unmethylated cytosines to

Table 1. Clinicopathologic Characteristics of Patients With IBDNs

	UC (n = 66)	CD (n = 8)	IC (n = 4)	Total (n = 78)
Sex (male/female)	37/29	4/4	2/2	43/35
Age (y)				
Mean \pm SD	49.8 \pm 16.3	49.1 \pm 15.3	43.8 \pm 14.0	49.3 \pm 15.9
Range	22–84	34–81	28–62	22–84
Extent of disease				
Proctitis	2	1	0	3
Left-sided	4	1	0	5
Pancolitis	40	4	3	47
Ileitis	0 ^a	2	0 ^a	2
Not known	19	0	1	20
Most advanced pathology				
Dysplasia	15	1	0	16
Cancer	51	7	4	62

^aThree patients with UC and 3 patients with IC had pancolitis with backwash ileitis.

uracils before methylation-specific polymerase chain reaction as described previously.⁵⁶ DNA methylation levels of genes were determined with real-time quantitative methylation-specific polymerase chain reaction using the ABI 7700 Sequence Detection (TaqMan) System (Applied Biosystems, Foster City, CA), as described previously.^{57,58} Primers and probes for quantitative methylation-specific polymerase chain reaction were as described for *MLH1*, *HPP1*, and *RAB32*.^{52,58,59} A normalized methylation value (NMV), reflecting the percentage of DNA methylated for the gene of interest (*GoI*), was defined as follows: $NMV = (GoI-S/GoI-FM)/(ACTB-S/ACTB-FM) \times 100$, where *GoI-S* and *GoI-FM* represent *GoI* methylation levels in the sample and fully methylated (*FM*) DNAs, respectively, whereas *ACTB-S* and *ACTB-FM* correspond to β -actin in the sample and fully methylated DNAs, respectively.

Statistical Analyses

Receiver operator characteristic curve analysis was performed using CpG island methylation data from non-dysplastic mucosae of patients with IBD and cancer specimens. Dichotomized variables were compared between different groups using Fisher exact test. Continuous variables were compared using Student *t* test. The Kruskal–Wallis test was used to test for significant differences in marker levels among multiple groups. All tests were 2 sided. *P* values less than .05 were considered statistically significant. Receiver operator characteristic curve analysis was performed using Analyse-it software (version 1.71; Analyse-it, Ltd, Leeds, England). For all other statistical calculations, the software package Statistica (version 6.1; StatSoft, Inc, Tulsa, OK) was used.

Results

Clinicopathologic Characteristics of Patients and Lesions

The clinicopathologic characteristics of all 78 patients are shown in Table 1. Sixty-two patients had a

carcinoma, and 16 patients had dysplastic lesions as their most advanced IBDN. Most patients had pancolitis. Two patients with CD had isolated ileitis and no colitis. No significant differences were observed in age or sex among the UC, CD, and IC groups.

Forty-three dysplasias were included, of which 41 were obtained from patients with UC. Thirty dysplasias were polypoid (DALM), and 12 were flat dysplasias. Ten dysplasias were high grade, and 26 were low grade (Table 2). Eighty-one carcinomas were included. Sixty-six were from patients with UC, 9 from patients with CD, and 6 from patients with IC. Localization and TNM stages are shown in Table 2. Two of 9 carcinomas from patients with CD were small bowel carcinomas. All remaining tumors and dysplasias were located in the colon or the rectum. Small bowel cancers occurred significantly more often in patients with CD compared with UC (*P* = .0153).

Prevalence of MSI-H in IBD-Associated Neoplasms

MSI-H was present in 19 (15%) of 124 IBDNs (13 cancers and 5 dysplasias from patients with UC, plus 1 cancer from 1 patient with IC; no CD lesions were MSI-H). The prevalence of MSI-H did not differ between dysplasias and cancers (12% vs 17%, respectively; *P* = .44). The rate of MSI-H in UC-associated CRC was 20% (Table 3).

No significant differences were observed among UC, CD, and IC (*P* = .266; Kruskal–Wallis test) or by pairwise comparison of UC, CD, and IC regarding the frequency of MSI-H versus non-MSI-H for all neoplasias, cancers, or dysplasias. When MSI-L and MSI-H were considered as a single group, a significant difference was observed between UC and CD versus MSS for all neo-

Table 2. Histopathologic Characteristics of IBDNs

Lesions	UC (n = 107)	CD (n = 10)	IC (n = 7)	Total (n = 124)
Dysplasia				
All	41	1	1	43
Grading				
Indefinite	1	0	0	1
Low-grade	26	0	1	27
High-grade	10	1	0	11
Not known	4	0	0	4
Morphology				
Flat	12	0	0	10
DALM	30	1	0	27
Not known	1	0	1	6
Cancer				
All	66	9	6	81
T stage				
Tx	16	0	0	16
Cis ^a	1	0	0	1
T1	7	0	1	8
T2	15	1	1	17
T3	24	6	4	34
T4	3	2	0	5
N stage				
Nx	18	0	0	18
N0	29	3	4	36
N1	12	3	1	16
N2	6	3	1	10
N3	1	0	0	1
Dukes'				
A	18	1	1	20
B	14	2	3	19
C	22	5	2	30
D	3	1	0	3
Not known	8	0	0	8
Localization				
Small bowel	0	2	0	2
Right colon	25	1	3	28
Left colon	21	3	3	27
Rectosigmoid/ rectum	14	3	0	17
Not known	6	0	0	6

^aCarcinoma in situ.

plastic lesions ($P < .005$), and a strong trend was also observed for cancers ($P = .06$).

Clinical Phenotype of MSI-H IBDNs Versus Non-IBD MSI-H CRCs

Of note, MSI-H IBDNs were not significantly more often right sided, were independent of histologic grade and stage, and had no sex or age association relative to non-MSI-H IBDNs (Table 4), although a nonsignificant tendency toward right-sided localization and male sex was observed in this group.

Compared with non-IBD MSI-H CRCs of mainly sporadic origin (36 sporadic CRCs and 10 HNPCC CRCs), we observed that IBD-associated CRCs were diagnosed at a younger age; however, this finding was independent of MSI status in patients with IBD ($P <$

.005 for MSI-H and non-MSI-H IBD CRCs). This difference was due to a late occurrence in the sporadic MSI-H group ($P < .0005$), whereas no such difference was observed between both IBD-CRC groups and HNPCC-associated CRCs. In addition, right-sided CRC location was significantly less frequent in MSI-H IBD CRCs than in sporadic MSI-H CRCs ($P < .05$). MSI-H IBD CRCs tended to be less frequent in female patients and more advanced and less often poorly differentiated compared with non-IBD MSI-H CRCs, but these differences did not reach statistical significance.

Profile of Frameshift Mutations in MSI-H Neoplasias

Fifteen MSI-H IBDNs (14 cancers and 1 DALM with high-grade dysplasia) were analyzed for frameshift mutations in 28 coding mononucleotide repeats and compared with a previously published cohort of 46 non-IBD MSI-H CRCs.⁵¹ The mutation frequency was not significantly different between these 2 groups for 25 of 28 genes studied. However, for *TGFBR2* and *ACVR2*, we observed a significantly lower frequency of mutations in IBDNs versus non-IBD CRCs (50% vs 79% [$P = .047$] and 21% vs 58% [$P = .029$], respectively). In contrast, the *ICAI* gene showed a significantly higher mutation frequency of 33% in IBDNs versus only 4% in non-IBD CRCs ($P = .028$). This same latter trend was observed for *SEC63*, although it failed to reach statistical significance ($P = .06$) (Table 5).

Methylation of *MLH1*, *HPP1*, and *RAB32*

Promoter methylation analysis was performed quantitatively using real-time methylation-specific polymerase chain reaction. NMVs for *MLH1*, *HPP1*, and

Table 3. MSI Status in IBDNs

	UC	CD	IC	Total
All neoplastic lesions				
All	107	10	7	124
MSI status (%)				
MSS	57 (53)	9 (90)	6 (86)	72 (58)
MSI-L	32 (30)	1 (10)	0 (0)	33 (27)
MSI-H	18 (17)	0 (0)	1 (14)	19 (15)
Dysplasia				
All	41	1	1	43
MSI status (%)				
MSS	23 (56)	1 (100)	1 (100)	25 (58)
MSI-L	13 (32)	0 (0)	0 (0)	13 (30)
MSI-H	5 (12)	0 (0)	0 (0)	5 (12)
Cancer				
All	66	9	6	81
MSI status (%)				
MSS	34 (51)	8 (89)	5 (83)	47 (58)
MSI-L	19 (29)	1 (11)	0 (0)	20 (25)
MSI-H	13 (20)	0 (0)	1 (17)	14 (17)

Table 4. Comparison of Clinicopathologic Variables of MSI-H and Non-MSI-H From IBD-Associated CRCs and Non-IBD MSI-H CRCs (36 Sporadic, 10 HNPCC)

	IBD-associated CRC		Non-IBD MSI-H CRC		
	MSI-H	Non-MSI-H	All	Sporadic	HNPCC
No. of patients	12	60	44	35	9
Age (y)					
Mean \pm SD	47.4 \pm 14.7 ^{a,b}	49.5 \pm 14.4 ^{a,b}	65.9 \pm 13.1	70.9 \pm 8.5	46.4 \pm 9.2
Range	22–74	28–84	36–87	51–87	36–65
Sex					
Male/female	8/4	31/29	17/27	12/23	5/4
% Female	33 ^c	48 ^c	61	66 ^c	44
No. of cancers	14	65	46	36	10
Localization					
Right/left	8/6	29/36	40/6	32/4	8/2
% Right sided	57 ^d	45 ^{e,f}	87	89	80
Dukes' stage					
Carcinoma in situ	0	0	1	0	1
A	4	12	4	4	0
B	5	22	29	23	6
C	4	26	8	7	1
D	1	1	2	1	1
Not known	0	4	2	1	1
% Advanced (C/D)	38	44 ^g	23	23	22
Grading					
Well	3	15	2	2	0
Moderate	5	25	15	12	3
Poor	4	22	16	13	3
Not known	2	3	12	9	3
Not applicable	0	0	1	0	1
% Poor	33	35	48	48	50

NOTE. Right sided indicates location between the cecum and the splenic flexure.

^a*P* < .005 compared with all non-IBD MSI-H CRCs.

^b*P* < .0005 compared with sporadic MSI-H CRCs.

^c*P* < .005 for MSI-H and non-MSI-H IBD CRCs vs non-IBD MSI-H CRCs.

^d*P* < .05 compared with all non-IBD MSI-H CRCs and sporadic MSI-H CRCs.

^e*P* < .0001 compared with all non-IBD MSI-H CRCs or with sporadic MSI-H CRCs.

^f*P* < .05 compared with HNPCC CRCs.

^g*P* < .01 compared with all non-IBD MSI-H CRCs or with sporadic MSI-H CRCs.

RAB32 in nondysplastic colon, dysplasia, and cancers from patients with IBD were calculated for each sample, as shown in Figure 1.

HPP1 methylation occurred early and at similar levels in dysplasias and cancers, and methylation levels were significantly higher in these tissues than in nondysplastic mucosa (Figure 1). For *RAB32*, we observed a nonsignificant trend for mean NMVs in dysplasias versus cancers (*P* = .10), suggesting that *RAB32* methylation occurs at the transition from dysplasia to carcinoma (Figure 1). No significant difference was observed in mean NMVs of MSS, MSI-L, and MSI-H lesions for *HPP1* and *RAB32* (data not shown). For *MLH1*, MSI-H IBDNs (n = 10) revealed a significantly higher mean NMV than did MSS IBDNs (n = 72) (*P* < .00001; (Figure 2). A strong but nonsignificant trend was observed when comparing MSS and MSI-L IBDNs (n = 17) (*P* = .10).

Discussion

In patients with IBD, CRCs arise in a field of chronically inflamed mucosa with multiple somatic alterations, exemplified by aneuploidy, loss of heterozygosity, hypermethylation, and *p53* mutations. IBDNs and sporadic CRC appear to harbor somewhat differing clinical phenotypes and molecular genetics, particularly regarding the frequency and timing of gene alterations.

Previous studies have reported a wide range of MSI-H frequency in IBDNs (<1% to 45%).^{36,44–50} Several factors may have contributed to this discrepancy. Firstly, microsatellite marker panels, the number of microsatellite markers used, and classifications of MSI have varied widely among different studies. A number of studies were performed before the development of a consensus definition of MSI, especially in distinguishing MSI-L from MSI-H. In the current study, all samples were

Table 5. Mutations of Coding Mononucleotide Repeats in Colorectal Tumors With MSI-H: Non-IBD-Associated Colorectal Cancers Versus IBDNs

Gene	Non-IBD MSI-H CRC			MSI-H IBDNs			P
	Not mutated	Mutated	(%)	Not mutated	Mutated	(%)	
TGFB2	9	34	79	7	7	50	.047
ACVR2	18	25	58	11	3	21	.03
SEC63	22	21	49	3	12	80	.06
AIM2	22	20	48	8	7	47	1.00
BAX	26	15	37	12	2	14	.18
NDUFC2	31	12	28	7	2	22	1.00
TTK	32	12	27	4	5	56	.12
MSH3	31	11	26	8	7	47	.19
KIAA0977	32	10	24	8	1	11	.66
DD5	33	10	23	8	1	11	.66
PA2G4	34	9	21	7	2	22	1.00
MSH6	37	6	14	14	1	7	.66
IGF2R	38	5	12	14	1	7	1.00
RAB2L	38	5	12	9	0	0	.57
ZMPSTE24	34	3	8	8	1	11	1.00
P4HB	39	3	7	9	0	0	1.00
BLM	40	3	7	9	0	0	1.00
PRKCBP1	41	3	7	9	0	0	1.00
CANX	39	2	5	7	2	22	.14
BCL10	43	2	4	9	0	0	1.00
PRDM2	42	1	2	8	1	11	.31
ICA1	43	2	4	6	3	33	.03
CASP1	40	1	2	9	0	0	1.00
RNF103	42	1	2	9	0	0	1.00
HNRPH1	41	0	0	8	1	11	.18
NFRKB	44	0	0	9	0	0	—
MK167	45	0	0	9	0	0	—

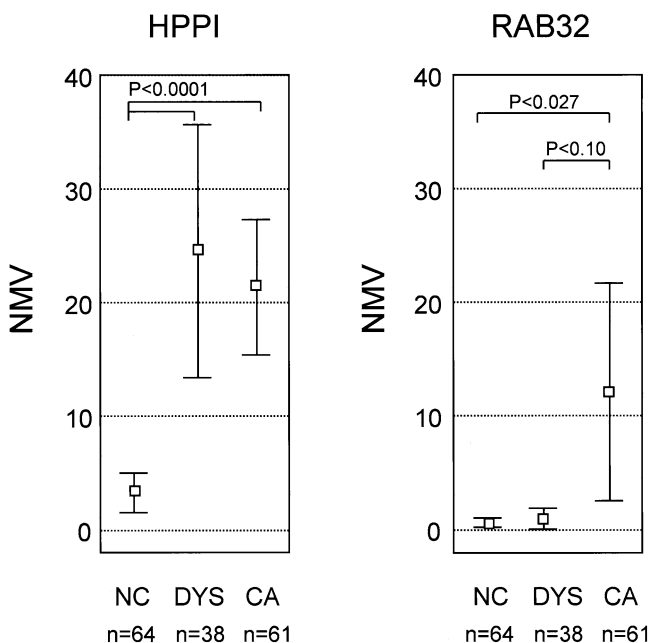


Figure 1. Mean NMV and the corresponding 95% confidence interval of *MLH1*, *HPP1*, and *RAB32* in nondysplastic colon (NC), dysplasia (DYS), and cancers (CA) from patients with IBD. *HPP1* methylation occurred at similar levels in dysplasias and cancers and at significantly higher rates than in nondysplastic mucosa. *RAB32* methylation occurred only in carcinoma, not being detectable in nondysplastic or dysplastic mucosa.

analyzed using the reference NIH consensus marker panel and were classified as recommended.⁵⁵ Secondly, the frequency of MSI may depend on the source of control tissue. Inflamed colonic mucosa may display MSI itself and therefore reduce the observed frequency of MSI in IBDNs.^{48,60} Therefore, in the current study, we used uninflamed mucosa from the terminal ileum in all but 4 cases. In addition, previously reported studies were performed in different geographic areas with differing ethnic backgrounds and contributory dietary factors. For example, folate status and polymorphisms of folate metabolism-associated enzymes have been associated with MSI-H.⁶¹ The current study was performed in a homogeneous cohort of patients from North America composed mostly of Caucasians and representative of the IBD patient population in the United States as a whole.

In the current study, we showed that the prevalence of MSI-H is similar between IBDNs and sporadic CRCs (15%–20%). In these patients with IBDN, we did not detect a specific clinical phenotype, such as right-sided predominance of MSI-H lesions, association with lower differentiation grade, or female sex, all of which are characteristic of sporadic MSI-H CRCs.^{62–64} We observed a significantly earlier age at diagnosis in IBDNs

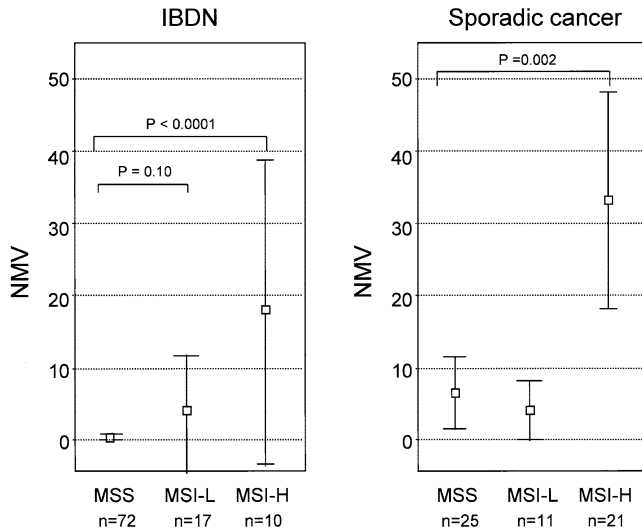


Figure 2. Mean NMVs and corresponding 95% confidence intervals of *MLH1* methylation correlated with MSI status in IBDNs (left) and non-IBD-associated CRCs (right; data taken from Mori et al⁵²). *MLH1* methylation values were significantly higher in MSI-H lesions compared with MSS lesions in both IBDN and sporadic groups.

compared with non-IBD CRCs, but this was independent of MSI status. We observed a relatively high frequency of MSI-L in IBDNs, particularly in UC (30%). The DNA mismatch repair (MMR) system corrects single-base pair mismatches and small insertion/deletion loops that occur during DNA replication. MSI-L is seen in some chronically inflamed tissues in the absence of genetic inactivation of the MMR system.^{48,65} It was previously shown that noncytotoxic levels of H₂O₂ inactivate both single base-pair mismatch and loop repair activities of the MMR system in a dose-dependent fashion and that this inactivation is most likely due to oxidative damage hMutS α , hMutS β , and hMutL α protein complexes.⁶⁶ Thus, reduced MMR activity may contribute to the high proportion of MSI-L lesions observed in inflammatory lesions, such as the IBDNs in our series.

The instabilotype, or the comprehensive profiling of coding region microsatellite mutations, of MSI-H IBDNs has not been investigated extensively in previous studies. We previously reported frameshift mutations of *TGFBR2*, *IGFR2*, and *E2F4* in 18%, 6%, and 33% of a small group of MSI-H IBDNs.⁶⁷⁻⁷⁰ We and others recently described the instabilotype of sporadic or hereditary MSI-H cancers, mainly of the colon, stomach, and endometrium.^{51,54,71-75} In the current study, we showed that the coding region mutational spectra of MSI-H IBDNs and non-IBD MSI-H CRCs differ significantly at 3 specific genetic loci but are similar at 25 other loci. *TGFBR2* was mutated in 50% of MSI-H IBDNs versus 79% of non-IBD MSI-H CRCs, confirming our previous

findings.^{67,70} A novel finding was the less frequent mutation of *ACVR2* in IBDNs than in non-IBD MSI-H CRCs. *ACVR2* is a putative tumor suppressor gene that is frequently mutated and results in silencing of gene expression in sporadic MSI-H colon and gastric cancers^{51,54,76} and in HNPCC-associated small bowel carcinomas.⁷⁷ In addition, mutations of *ACVR2* and loss of heterozygosity at the *ACVR2* locus in non-MSI CRCs have been described.⁷⁸ *ACVR2* is a member of the transforming growth factor β receptor family and controls cell growth and differentiation. SMAD proteins are major intracellular effectors shared by *ACVR2* and *TGFBR2* signaling. Most recently, we have shown that the restoration of wild-type *ACVR2* in a natively *ACVR2*-deficient MSI-H colon cancer cell line resulted in cell-growth suppression, restoration of the SMAD4 signaling, and overexpression of genes implicated in the control of cell growth and tumorigenesis that are shared with *TGFBR2* activation.⁷⁹ The significantly lower frequency of *TGFBR2* and *ACVR2* mutations in IBDNs may emphasize the importance of other genetic alterations in the SMAD signaling pathway, for example, *SMAD4* inactivation, which is supported by a high loss of heterozygosity rate on chromosome 18q21 in UC-associated cancers.^{21,23,80} However, *SMAD4* mutations have not been frequently detected in IBDNs.^{38,39} Recently, methylation of *SMAD8* was reported in approximately one third of CRCs.⁸¹ Moreover, methylation of *TGFBR1* has been reported in 13%–50% of sporadic gastric cancers.^{82,83} Thus, the SMAD pathways appear to be important in this context. In addition, the low *TGFBR2* and *ACVR2* mutation prevalence in IBDNs may be explained by the fact that IBD mucosa already has low innate TGF- β responsiveness.^{84,85} Thus, if this responsiveness is already low, there may be less need for additional frameshift mutations than in sporadic carcinogenesis.

The highly conserved *ICA1* gene is located at chromosome 7p22 and encodes a 69-kilodalton autoantigen (ICA69). ICA69 is one target of the immune processes defining the pathogenesis of type 1 diabetes.⁸⁶⁻⁸⁹ The cellular function of ICA69 is unknown so far. Recently, it has been shown that three 5'-untranslated region exons are expressed in a tissue-specific manner and that the *ICA1* promoter contains a CpG island. However, thus far, *ICA1* has not been linked to carcinogenesis.

Multiple molecular differences have been observed between IBD-associated and sporadic colorectal neoplasms. Examples include *K-ras* and *APC* gene mutation (rare in the former, common in the latter) and *p53* mutation (occurring early in the former, late in the latter).^{13,26,30-44} Moreover, there seem to be biologic differences between the 2 forms of neoplasia. Progress-

sion in IBD appears to be more rapid, occurring from dysplasia to cancer over a few years; in the sporadic polyp-carcinoma sequence, the progression period appears to be more on the order of 15 years. Cancers in IBD often arise from flat dysplastic lesions or DALMs, whereas cancers in the sporadic pathway often derive from polypoid adenomas.¹² These and many other biologic, clinical, and molecular differences may reflect, or be reflected by, the unique mutational spectrum observed for IBDNs.

Promoter hypermethylation, the epigenetic addition of methyl groups to DNA at CpG islands, has recently been established as a common mechanism of gene inactivation in human carcinogenesis.^{90–92} Certain methylation events can occur with aging or inflammation, conditions themselves associated with increased neoplastic risk.^{93–96}

The observed lack of increase in *MLH1* hypermethylation during progression of IBDNs may be due to the low frequency of MSI-H (12%–17% in dysplasias and cancers). In addition, *MLH1* hypermethylation may represent an early step in carcinogenesis in a subset of IBDNs, occurring even in nondysplastic mucosa. The relatively low NMV of *MLH1* resulted from a lack of methylation for most of the IBD lesions.

Whereas sporadic MSI-H CRCs are closely related to methylation and resultant gene silencing of *MLH1*,^{97–99} other mechanisms underlying MSI may be important in IBDNs. It has been shown that overexpression of DNA polymerase β causes MSI in vitro.¹⁰⁰ Recently, the development of MSI in inflamed nonneoplastic UC specimens was linked to overexpression of BER proteins as a consequence of free radical overload caused by inflammation.⁶⁰ However, the exact mechanism explaining how BER contributes to MSI remains to be elucidated. Regarding the minority of MSI-H IBDNs that do not have *MLH1* hypermethylation, the explanation could be either concurrent IBDN and HNPCC or, more likely, de novo sporadic MMR gene mutation due to an increased mutation frequency caused by inflammation (plus or minus loss of heterozygosity). A total of 10%–30% of sporadic MSI-H CRCs are negative for *MLH1* hypermethylation. Previous data showed that non-*MLH1* methylated MSI-H tumors are likely to carry somatic mutations¹⁰¹; therefore, a similar event is likely in MSI-H IBDNs. Other possible explanations include mutations in other causative genes for MSI-H (ie, genes other than *MLH1*, such as *MSH2*, *MSH6*, *MLH3*, *MSH3*, *PMS1*, and *PMS2*).

We have previously reported that methylation of *MLH1* occurred only in gastric cancers with *HPP1* methylation, suggesting earlier involvement of *HPP1* than of

MLH1 in the pathogenesis of MSI-H gastric cancers.⁵⁷ We also found that methylation of *HPP1* is an early event in the pathogenesis of UC-associated dysplasias and carcinomas, but not in nonneoplastic mucosae, and that methylation of *HPP1* causes gene silencing.⁵⁸ Most recently, we identified *HPP1* methylation as a frequent and early event in the progression of Barrett's esophagus to esophageal adenocarcinoma.¹⁰² In the current study, we discovered that *HPP1* methylation is not linked to MSI-H in IBDNs. In contrast to the early occurrence of *HPP1* methylation in IBDNs, we showed that *RAB32* methylation occurs late, at the transition to invasive carcinoma. We have previously shown a correlation between *RAB32* hypermethylation and messenger RNA expression levels in MSI-H primary CRCs and CRC cell lines.⁵² Similarly to *HPP1*, *RAB32* methylation was not linked to MSI-H in IBDNs.

In summary, these findings suggest that altered methylation occurs early in IBD-associated lesions and that a unique pattern of coding region microsatellite mutations (instabilotype) distinguishes IBDNs from their sporadic CRC counterparts. These discoveries have important implications regarding the early detection of IBD-associated cancer and in distinguishing IBD-associated lesions, along with their attendant underlying molecular pathways, from sporadic colon neoplasms.

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Peyer of Peyer's Patches

Johann Conrad Peyer (1653–1712) was born at Schaffhausen, in view of the Rheinfall in northern Switzerland. A student of Duvernoy in Paris, he returned to the university in his native town as professor of rhetoric, logic, and medicine. Peyer claimed to have first observed follicular aggregates in the mucosa of the small intestine in 1673 but did not publish his findings until 1677. It is doubtful that Peyer had access to microscopy (which was introduced by Anton van Leeuwenhoek about that same time). Peyer supposed the “patches” secreted a digestive juice. Only later were the aggregates defined as lymphoid tissue and recognized as a component of the enteric immune system.

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