Biology of Human Tumors

Clinical Cancer Research

Excess of Proximal Microsatellite-Stable Colorectal Cancer in African Americans from a Multiethnic Study

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Abstract

Purpose: African Americans (AA) have the highest incidence of colorectal cancer compared with other U.S. populations and more proximal colorectal cancers. The objective is to elucidate the basis of these cancer disparities.

Experimental design: Of note, 566 AA and 328 non-Hispanic White (NHW) colorectal cancers were ascertained in five Chicago hospitals. Clinical and exposure data were collected. Microsatellite instability (MSI) and *BRAF* (V600E) and *KRAS* mutations were tested. Statistical significance of categorical variables was tested by the Fisher exact test or logistic regression and age by the Mann–Whitney *U* test.

Results: Over a 10-year period, the median age at diagnosis significantly decreased for both AAs (68–61; $P < 0.01$) and NHWs (64.5– 62; $P = 0.04$); more AA patients were diagnosed before age 50 than NHWs (22% vs. 15%; $P = 0.01$). AAs had more proximal colorectal cancer than NHWs (49.5% vs. 33.7%; $P < 0.01$), but overall frequencies of MSI, *BRAF* and *KRAS* mutations were not different nor were they different by location in the colon. Proximal colorectal cancers often presented with lymphocytic infiltrate ($P < 0.01$) and were diagnosed at older ages ($P = 0.02$). Smoking, drinking, and obesity were less common in this group, but results were not statistically significant.

Conclusions: Patients with colorectal cancer have gotten progressively younger. The excess of colorectal cancer in AAs predominantly consists of more proximal, microsatellite stable tumors, commonly presenting lymphocytic infiltrate and less often associated with toxic exposures or a higher BMI. Younger AAs had more distal colorectal cancers than older ones. These data suggest two different mechanisms driving younger age and proximal location of colorectal cancers in AAs. *Clin Cancer Res; 20(18); 4962–70. ©2014 AACR.*

Introduction

Colorectal cancer represented 9% of all diagnosed cancers in the United States in 2012 (1) but incidence rates had started a slow and steady decline almost 30 years ago, even before the generalization of colorectal cancer screening (2). In contrast with the general decline, Surveillance Epidemiology and End Results (SEER) data have shown that since 1992 colorectal cancer incidence rates are increasing among adults younger than 50 (3). Some studies have suggested that young-onset colorectal cancer seems to disproportionally affect non-White, underinsured patients who live in southern and western parts of the United States (4, 5) Because most adults younger than age 50 are not screened for colorectal cancer, the shift toward younger ages at diagnosis very likely is not be explained by earlier detection. The lower mean age of presentation in African Americans (AA) has prompted some medical organizations to recommend colorectal cancer screening for average risk AAs to be started at a younger age than the current recommendation of age 50 in non-Hispanic Whites (NHW; ref. 6).

Another important difference between AA and NHW patients with colorectal cancer is the higher incidence of proximal adenomas and cancers (defined here as tumors proximal to the splenic flexure) documented in AAs over the last 30 years (7, 8). The site of tumor development in colorectal cancer has important implications not only related to screening but also due to the distinct biologic features and prognosis (9). For example, death from proximal colorectal cancers seems to be less preventable

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Translational Relevance

African Americans (AA) disproportionately die from colorectal cancer, and health disparities compared with Whites are not well understood. We show that a very significant number of AAs are diagnosed before age 50 compared with Whites, and their tumors are usually more advanced at diagnosis. Furthermore, in AAs there is a significant excess of proximal colorectal cancers, consisting of microsatellite stable tumors commonly exhibiting lymphocytic infiltrate and less often associated with smoking, drinking, or higher BMI. Because proximal colorectal cancers are associated with higher missed tumor rates on colonoscopy and increased risk of interval cancers, we believe that clinical practice should be changed to screen AAs at earlier ages and to lower the threshold for performing diagnostic tests when suspicious symptoms present in young AAs. Specific screening and diagnostic approaches will help eliminate colorectal cancer health disparities in AAs.

by colonoscopy performance (10) and this outcome could be related to higher miss rates of proximal lesions (11). Missed tumors as well as lower screening rates could also contribute to the reported more advanced stages at presentation of colorectal cancer in AAs compared with NHWs (7).

Significant biologic differences exist between proximal and distal colorectal cancers, including a higher percentage of tumors with microsatellite instability (MSI), increased hypermethylation, and increased gene mutation rates in proximal tumors (12). These differences could have prognostic and therapeutic implications. In this regard, some authors have suggested the presence of higher MSI rates and CpG island hypermethylation in AAs (13). Environmental factors such as diet could also play a role in colorectal cancer differences; for example, higher red blood cell folate levels have been associated with increased methylation of genes that control colonic growth and cell differentiation in tumors from AAs (14).

A higher frequency of MSI in AA colorectal cancers might explain the increased proportion of proximal colorectal cancers in this population (15, 16); however, MSI frequency in AA colorectal cancer has not been well determined, as studies have been limited by relatively small samples sizes (16–19). To address this limitation, we investigated the presence of MSI as a potential explanation for the excess proximal colorectal cancer in AAs in a large collection of unselected cases. We addressed the MSI hypothesis and related questions in cases from the Chicago Colorectal Cancer Consortium (CCCC)—a multi-institutional study of colorectal cancer in an ethnically diverse urban area. The present report from the CCCC is the most comprehensive one to date that compares differences in key clinical and molecular features in AA and NHW colorectal cancers living in a single geographic area.

Materials and Methods

Ascertainment, recruitment, and study design

The CCCC includes five large Chicago medical centers: the University of Illinois Hospital and Health Sciences System (UIHHS), Jesse Brown Veterans Administration (JBVA), John H. Stroger Hospital of Cook County (JHSHCC), University of Chicago Medicine, and Rush University Medical Center. The CCCC ascertained patients with incident colorectal cancer from search of Pathology records (the majority of cases coming from 1997–2010) and from prospective enrolment in surgery and endoscopy units (2011–2012). Patients with colorectal cancer recurrence; inflammatory bowel disease; non-adenocarcinoma tumors were excluded. Patients with unspecified tumor locations or multiple primaries were excluded from the analysis.

From this ascertainment, we were able to include 894 patients with colorectal cancer (566 AAs and 328 NHWs). Other ethnic groups were not included. Biologic samples were available from 635 individuals: 409 AAs and 228 NHWs. For every type of analysis, the number of patients that were available and informative for analysis is expressed as the denominator in every cell of the tables. The median age at diagnosis was 63, and 56.7% were male and 43.3% female.

For all cases, we collected data from pathology, radiology, endoscopy, clinical, and operative reports. Cancer staging was determined according to criteria set by the American Joint Committee on Cancer staging system (20). Mucinous phenotype was considered positive when more than 50% of the tumor displayed mucin production (20).

For the subset of cases that were prospectively enrolled, we administered an extensive personal questionnaire. The questionnaire collected information on demographics and socio-economic data, medical and family history of cancer (traced backward and laterally at least up to second-degree relatives), smoking and alcohol consumption, use of medications and supplements over the previous 5 years, and physical exercise. Significant exercise constituted at least 150 minutes a week of moderate intensity exercise or 75 minutes a week of vigorous intensity exercise as determined by the 2008 Physical Activity Guidelines for Americans (21). Obesity was assessed by the body mass index (BMI; ref. 21). The study was conducted according to the corresponding approved Institutional Review Board protocol at each institution.

Biospecimen collection

For cases ascertained through searches of pathology records, paraffin blocks, and slides were pulled from the Pathology Department archives. Areas of tumor and non-tumor tissue were identified and cores were collected. Paraffin was removed using an octane/methanol method (22). DNA was then prepared using the Gentra Puregene DNA Isolation Kit (Qiagen) according to the manufacturer's instructions, except the proteinase K extraction step was extended to 3 days, adding fresh enzyme on each day, and the sample was heated at 95ºC for 15 minutes before

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protein precipitation. For cases ascertained in surgery or endoscopy, fresh biopsies were taken from tumors and uninvolved colonic mucosa 10 cm away from the tumor. Biopsies were preserved in RNAlater (Life Technologies Corporation) buffer and frozen. DNAs were extracted from ground tissue using the Maxwell 16 Tissue DNA Purification Kit (Promega).

Molecular analysis

MSI was assessed in paired DNA samples from tumor and uninvolved tissue. The panel of mononucleotide markers included NR21, NR22, NR24, NR27, BAT25, and BAT26 (23). The use of a mononuclotide panel has been shown to be superior for MSI detection than the NCI panel (24). Multiplex PCR amplified all markers, and PCR products were analyzed by capillary electrophoresis, as previously described (24). The most common mutations of *BRAF* (V600E; ref. 25) and *KRAS* (codons 12 and 13; ref. 26) were analyzed through direct DNA sequencing. Amplification and sequencing of the candidate regions were performed as previously described (27).

Illinois State Cancer Registry data

Chicago is part of Cook County, the second most populous county in the United States with 5,231,351 residents. The Illinois State Cancer Registry (ISCR) collects statewide cancer data through mandated reporting by medical centers, pathology laboratories, and through data exchange with other states. Cook County colorectal cancer incidence and staging data were obtained from the publicly available dataset of the ISCR (28). AA and NHW patients with colorectal cancer registered between 1991 and 2010 (3,553 AAs and 10,247 NHWs colorectal cancer cases) were analyzed by age of diagnosis and cancer stage.

Statistical analysis

Differences in categorical variables were assessed by the Fisher exact test or χ^2 test. Differences in age were analyzed by the Mann–Whitney *U* test. We performed a primary analysis on all cases for which data were available in more than 80% of individuals. Primary analysis included age, sex, histologic grade, and MSI. We performed a secondary analysis on the subset of cases from which data were available through the administration of the personal questionnaire and their medical records.

To identify factors associated with proximal tumor location in AAs, we performed logistic regression that included the following covariates: first-degree relative with colorectal cancer; previous colonoscopy; previous colon polyps; exercise; smoking (packs/year); alcohol (g/d); use of aspirin, NSAIDs, COX2 inhibitors, and statins; mucinous phenotype; BMI; tumor stage; lymphocytic infiltrate, histologic grade and age. Before performing the logistic regression, to include all AA patients in the analysis, we used the Multivariate Imputations by Chained Equations (MICE) procedure to impute missing data based on the set of patients with available data. Normally distributed variables were imputed using predictive mean matching, binary variables by logistic regression, and categorical variables with >2 levels by polytomous logistic regression. Final estimates of ORs, 95% confidence intervals (CI), and *P* values were calculated by averaging statistics across the 50 complete datasets that we imputed and computing total variance by Rubin rules (29). The MICE package in R was used to perform the logistic regression on imputed data (30). All reported *P* values correspond to two-sided tests. Differences were considered statistically significant if the *P* value was less than 0.05. All statistical analyses were carried out using R 3.0.0 (31).

Results

Age and stage at diagnosis of colorectal cancer

To determine whether the change in the age distribution of colorectal cancer cases in the Chicago population is similar to the change observed in the general U.S. population, we compared the median age of diagnosis in the group of patients ascertained prospectively (2011–2012) with a similarly sized group of patients diagnosed with colorectal cancer 10 years ago (2000–2002). Patients in both ethnic groups diagnosed with colorectal cancer in 2011–2012 had a significantly lower median age at diagnosis than those diagnosed 10 years ago: 61 versus 68 for AAs ($P < 0.01$) and 62 versus 64.5 for NHWs ($P = 0.04$; Table 1). Over this period, the percentage of patients diagnosed with colorectal cancer before age 50 went from 11% to 22% in AAs but the percentage was not different in NHWs (Table 1). To determine whether the shift to younger ages at diagnosis could be related to earlier detection, we compared cancer stage distribution between the 2000 to 2002 and 2011 to 2012 groups. In the 2011 to 2012 groups, there was a 3% and 5% increase in cases with early-stage colorectal cancer (0–II) in AAs and NHWs, respectively, although these comparisons did not reach statistical significance (Table 1). The shift toward earlier ages of diagnosis was also observed and found to be significant in the colorectal cancer data for Cook County extracted from the ISCR (Supplementary Fig. S1 and Supplementary Table S1), indicating that the shift is not restricted to the hospitals sampled in the CCCC. The median ages of diagnosis of the 2011 to 2012 CCCC cases were lower than the median age data from the most recent SEER (2005–2009) nationwide survey, which showed median ages of 65 for AAs and 70 for NHWs (32).

Features that distinguish AA colorectal cancer by age

After observing the high percentage of young AA patients with colorectal cancer, we explored factors that could associate with younger age at diagnosis. AA patients, 50 years and younger were evenly distributed by sex as opposed to older patients that showed a male predominance similar to what is reported for colorectal cancer as a whole (57%; Table 2). Although the presence of a higher proportion of younger patients in the AA population could suggest a bigger genetic component, the secondary analysis showed that younger patients did not have more first-degree relatives with colorectal cancer ($P = 1$; Table 2). Colorectal cancers from younger patients tended to have more lymphocytic infiltrate than older patients (42% vs. 23%; $P = 0.08$) and more

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Table 1. Comparison of colorectal cancer cases within AAs and NHWs

	Years 2000—2002		Years 2011—2012		
	Percentage	***n* = 157**	**Percentage**	***n* = 137**	***P***
AAs					
Median age at diagnosis	68		61		<0.01
Individuals diagnosed at age 50 or younger	11%	17/157	22%	30/137	0.01
Cancer stage					0.71
0, I, II	48%	64/132	51%	66/129	
III, IV	52%	68/132	49%	63/129	
	Percentage	***n* = 102**	**Percentage**	***n* = 69**	***P***
NHWs					
Median age at diagnosis	64.5		62		0.04
Individuals diagnosed at age 50 or younger	14%	14/102	15%	10/69	1.00
Cancer stage					0.51
0, I, II	52%	45/87	57%	35/61	
III, IV	48%	42/87	43%	26/61	

advanced cancer stages (61% vs. 49.0; $P = 0.15$). No differences were seen in histologic grade or mucinous phenotype. The frequencies of MSI and *KRAS* mutations were similar in both age groups but no *BRAF* mutations were found in the younger AAs (Table 2). Only 3% of younger patients had a colonoscopy before diagnosis versus 27% of the older patients ($P < 0.01$; Table 2).

Clinical and molecular features that distinguish AA and NHW colorectal cancer

Some studies have reported significant differences in some tumor features between AAs and NHWs, such as lower histologic grade (7, 33), more advanced stages (7), and more proximal tumors in AAs (7). We did not see any differences in histologic grade, but AAs had a significantly

Table 2. Features of colorectal cancers by age at diagnosis in AAs only

	50 and Younger		Older than 50		*P*
Primary analysis					
Male	48%	32/66	57%	191/333	0.22
Proximal location	44%	28/64	51%	165/324	0.34
Histologic grade					0.80
Low	25%	14/55	22%	60/274	
Moderate	64%	35/55	65%	177/274	
High	11%	6/55	13%	37/274	
MSI	8%	5/67	9%	32/340	0.82
	50 and Younger		**Older than 50**		***P***
Secondary analysis					
Previous colonoscopy	3%	1/30	27%	28/105	<0.01
First-degree relative with colorectal cancer	13%	4/30	13%	13/100	1.00
Presence of lymphocytic infiltrate	42%	11/26	23%	26/112	0.08
Mucinous phenotype	13%	5/38	9%	14/158	0.50
Cancer stage					0.15
0, I, II	39%	19/49	51%	104/204	
III, IV	61%	30/49	49%	100/204	
BRAF V600E	0%	0/68	5%	16/340	0.08
KRAS (codons 12,13)	21%	7/34	23%	37/158	0.82

NOTE: Histologic grade was described as low (well to moderately differentiated), moderate (moderately differentiated), and high grade (poorly differentiated or undifferentiated). Patients with synchronous cancers were excluded from the tumor location comparison. Lymphocytic infiltrate was considered positive when mild, moderate, or marked infiltrates were described by the pathologist. Cancer staging was determined according to criteria set by the American Joint Committee on Cancer staging system. Mucinous phenotype was considered positive when more than 50% of the tumor displayed mucin production.

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Table 3. Comparison of clinical and molecular characteristics of colorectal cancer cases between AAs and NHWs

	AAs		NHWs		*P*
Primary analysis					
Median age at diagnosis	63.9		62.6		0.34
Male	56%	224/401	58%	131/225	0.61
Proximal location	49%	193/390	34%	68/202	<0.01
Histologic grade					0.81
Low	23%	75/330	20%	25/192	
Moderate	64%	212/330	67%	128/192	
High	13%	43/330	13%	39/192	
All colorectal cancers					
MSI	9%	38/409	9%	20/226	0.89
Proximal colorectal cancers only					
MSI	14%	26/191	18%	12/68	0.43
Distal colorectal cancers only					
MSI	3%	7/197	4%	5/134	1
Secondary analysis					
Previous colonoscopy	21%	29/135	19%	14/72	0.85
Mucinous phenotype	10%	19/197	5%	4/74	0.51
Presence of lymphocytic infiltrate	29%	40/139	12%	7/56	0.02
Cancer stage					0.01
0, I, II	48%	123/254	63%	66/105	
III, IV	52%	131/254	37%	39/105	
All colorectal cancers					
BRAF V600E	4%	16/409	7%	15/226	0.18
KRAS (codons 12,13)	23%	44/194	15%	13/86	0.15
Proximal colorectal cancers only					
BRAF V600E	7%	13/192	13%	9/68	0.13
KRAS (codons 12,13)	24%	20/82	25%	5/20	1.00

higher percentage of proximal tumors than NHWs (49.5% vs. 33.7%; $P = 0.01$; Table 3). Furthermore, from the secondary analysis there was a significantly higher frequency of advanced-stage tumors in AAs (52% in AAs vs. 37% in NHWs; $P = 0.01$) and tumor lymphocytic infiltrate was more common (29% in AA vs. 12% in NHWs; $P = 0.02$; Table 3). Data from the Cook County registry 2006 to 2010 also showed less localized and more metastatic colorectal cancers in AAs compared with NHWs (Supplementary Fig. S1B and Supplementary Table S1B).

Some authors have suggested that AA patients have much higher frequencies of MSI tumors than NHWs (13), which could explain the higher frequency of proximal colorectal cancers in AAs. We did not identify a significant difference in the percentage of colorectal cancer cases with MSI between AAs and NHWs (Table 3). Proximal colorectal cancers more often exhibited MSI than distal colorectal cancers in both AAs and NHWs, but MSI frequencies in proximal colorectal cancers were no different between the two ethnic groups ($P = 0.43$; Table 3). The frequencies of *KRAS* mutations in proximal colorectal cancers were similar in both ethnic groups, whereas *BRAF* mutations were less frequent in proximal colorectal cancers in AAs, but not significantly so. The frequencies of MSI in older and younger age groups were also similar ($P = 0.82$; Table 2).

Features that distinguish proximal and distal microsatellite stable colorectal cancers in AAs

To understand what biologic factors might be driving microsatellite stable (MSS) proximal colorectal cancers, we tested factors that could correlate with these colorectal cancers in AAs. Whereas male gender was not significantly associated with proximal MSS colorectal cancers ($P = 0.39$; Table 4), patients with proximal MSS colorectal cancers were older ($P = 0.02$; 4A). Younger patients had more distal than proximal colorectal cancers, though this difference was not significant (Table 4). In a multivariate analysis of variables from the secondary analysis, tumor lymphocytic infiltrate was independently associated with proximal location in MSS colorectal cancers in AAs (OR, 8.3; 95% CI, 1.11–62.30; $P = 0.04$). More proximal MSS colorectal cancers than distal MSS colorectal cancers were diagnosed at later stages ($P = 0.28$), despite the patients having undergone more previous colonoscopies ($P = 0.05$). On the other hand, a higher percentage of cases with distal MSS tumors were obese (BMI > 30 in 32% patients with distal

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Table 4. Features of MSS colorectal cancers by tumor location in AAs only

	Proximal location		Distal location		*P*
Primary analysis					
Median age at diagnosis	64.9		61.8		0.02
Individuals diagnosed at age 50 or younger	15%	25/165	18%	34/189	0.57
Individuals diagnosed at age 55 or younger	25%	42/165	29%	54/189	0.55
Male	60%	98/164	55%	101/185	0.39
Histologic grade					0.08
Low	28%	38/136	22%	33/151	
Moderate	59%	80/136	71%	107/151	
High	13%	18/136	7%	11/151	
Secondary analysis					
Obese (BMI > 30)	21%	13/62	32%	24/74	0.18
Significant exercise	24%	14/59	31%	22/71	0.43
Packs/year >0	49%	26/53	65%	46/71	0.14
Alcohol >0 g/d	20%	11/55	27%	18/67	0.18
Previous colonoscopy	29%	17/58	14%	10/70	0.05
First-degree relative with colorectal cancer	9%	5/54	16%	11/69	0.42
Aspirin/NSAIDs	71%	42/57	68%	48/71	0.71
Statins	25%	14/58	32%	23/71	0.43
Cox-2 inhibitors	7%	4/58	4%	3/71	0.70
Presence of lymphocytic infiltrate	44%	26/59	14%	9/66	<0.01
Mucinous phenotype	11%	8/74	8%	8/99	0.77
Cancer stage					0.28
0, I, II	42%	42/99	50%	63/125	
III, IV	58%	57/99	50%	62/125	
BRAF V600E	0%	0/165	1%	2/190	0.50
KRAS (codons 12,13)	26%	20/76	19%	18/95	0.27

NOTE: Patients with synchronous cancers were excluded. Cigarette consumption was assessed by pack-years: cigarettes smoked per day X years the person smoked. Alcohol consumption was recorded as mean of grams of alcohol consumed per day based on the content of each beverage. Significant exercise constituted at least 150 minutes a week of moderate intensity exercise or 75 minutes a week of vigorous intensity exercise as determined by the 2008 Physical Activity Guidelines for Americans.

colorectal cancer vs. 21% proximally), alcohol users (27% vs. 20.0%), and smokers (65% vs. 49%); however, none of these differences were statistically significant. Finally, the frequencies of *KRAS* and *BRAF* mutations were indistinguishable in proximal and distal MSS colorectal cancers (Table 4).

Discussion

Using patients ascertained through the CCCC, we collected samples and clinical data on an ethnically mixed population recruited in the same geographical area, allowing for a robust comparison over time and between AAs and NHWs—ethnic groups with a large disparity in both colorectal cancer incidence and colorectal cancer mortality. To our knowledge, this study includes the largest group of AA patients with colorectal cancer reported to date with not only granular clinical data but also relevant tumor molecular features.

The CCCC data collected present a dynamic picture of colorectal cancer that has evolved over a relatively short period of time toward a younger age at diagnosis in both AAs and NHWs, similar to what has been observed in the SEER registry (3). This shift in age of diagnosis is also observed in the ISCR data for Cook County in which 20% (773/3,878) of AAs and 14% (1,046/7,715) of NWHs were diagnosed before age 55 in the period 2006 to 2010 (Supplementary Fig. S1A and Supplementary Table S1A). A predominance of AAs among younger patients with colorectal cancer has also been reported within the National Cancer Database, a large hospital-based cancer registry (4).

Remarkably, although older patients are being diagnosed at earlier stages, the younger patients present with more advanced cancer. It is plausible that the widespread use of endoscopic procedures for either diagnostic purposes or secondary to implementation of screening strategies has contributed to the overall increase in detection of colorectal cancers at earlier stages. In fact, this trend has been recently shown in the National Bowel Cancer Screening Program in South Australia (34). Less clear is why colorectal cancer is affecting higher numbers of younger individuals (particularly AAs) and why these colorectal cancers are more often diagnosed when the cancer is more advanced. Younger ages

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at diagnosis could suggest a higher proportion of familial or syndromic cases; however, the frequency of younger AA cases with relatives with colorectal cancer was not increased, and the shift in age of diagnosis over such a short-time period is unlikely to be explained by genetic causes. Moreover, Lynch syndrome, the most common of all hereditary colorectal cancer syndromes, is associated with MSI and the frequency of MSI was not increased in younger AA colorectal cancer cases.

Given the shift toward earlier age of diagnosis, we believe it might be wise to evaluate the effectiveness of colorectal cancer screening strategies at younger ages, particularly in AAs. In fact, some authors have already suggested an earlier screening age in AAs (6). Furthermore, physicians may need to lower the threshold that prompts them to order diagnostic procedures in younger individuals with suspicious symptoms that due to their young age would not raise a high level of suspicion for colorectal malignancy.

One of the main goals of this study was to determine whether an increased frequency of MSI in AA colorectal cancer could explain the increased proportion of proximal colorectal cancers in this population. Our data provided a strong counterpoise to this hypothetical explanation. With 409 AA and 226 NHW colorectal cancers assayed, the frequency of MSI colorectal cancer in AAs was found to be no different from the frequency in the NHWs. In fact, other studies (17, 19) that also compared AAs and NHWs failed to find differences in MSI frequencies between AAs and NHWs (Supplementary Table S2). Moreover, when our data are combined with all the available studies, the MSI frequencies between AAs and NHWs are nearly identical to each other (Supplementary Table S2). The differences in frequencies across the various studies could reflect differences in biologic factors that underlie MSI (e.g., age, gender, and environmental triggers); alternatively, and not exclusively, as the frequency of MSI is low, the differences could reflect the vagaries of sampling. The MSI frequency in the present report is almost identical to that found in the Epicolon study, based on 1,200 consecutive patients from Spain (24). The robustness of our MSI testing methodology was asserted in that study as we showed an extremely high level of concordance between presence of MSI and loss of expression of mismatch repair proteins (24). Finally, it is worth noting that the study that showed the highest incidence of MSI in tumors from AA patients was based on limited number of cases (15).

Similarly, we did not see any significant differences in mutational rates of the commonly mutated MAPK genes, *KRAS* and *BRAF*, between AAs and NHWs. Neither did we observe a predominance of *KRAS* codon 13 mutations in proximal MSS tumors in AAs, as previously reported (17).

We did observe biologic differences in AA colorectal cancer compared with NHW colorectal cancer, characterized primarily by more proximal tumors, which has been observed in many studies of AA colorectal cancer. As noted above, these proximal tumors in AAs do have the same percentage of MSI as NHWs. Because the tumors on the right side still have a relatively low percentage of MSI, the great majority of right-sided tumors are MSS in both ethnic groups. Thus, in terms of percentages, AAs have many more right-sided MSS tumors than NHWs. In addition, this group of proximal MSS tumors in AAs is characterized by presence of lymphocytic infiltrate. Altogether, we find that the overall excess of colorectal cancers in AAs is mostly contributed by a higher prevalence of the proximal MSS phenotype. $CD8^+$-type lymphocytic infiltrations have been associated with both MSI and MSS colorectal cancers (35). Elevated MSI at selected tetranucleotide repeats (EMAST) in MSS colorectal cancers has been linked to inflammatory processes in the tumor and heterogeneous expression of the DNA mismatch repair protein MSH3 (36). It is possible that the excess proximal MSS, inflammatory colorectal cancers seen in AAs is related in some way to the EMAST phenomenon. Further studies are warranted to test whether specific lymphocyte and novel genomic-instability phenotypes are associated with proximal MSS colorectal cancers in AAs.

Ferracin and colleagues reported the association of a subgroup of proximal MSS cancers with *BRAF* mutations, CpG island methylator phenotype (CIMP), mucinous phenotype, chromosomal instability (CIN), and a unique gene-expression profile (37)—a pattern of clinical correlations that is similar to those observed in MSI tumors. These observations suggest that a subset of proximal tumors originate through the methylator phenotype but only those with *MLH1* promoter methylation develop MSI. Bond and colleagues (38) suggested that MSS cancers with *BRAF* mutations are fundamentally different from MSI/*BRAF*–mutated cancers but that both types of tumors preferentially develop in the proximal colon. MSS/*BRAF*–mutated colorectal cancers were found to have levels of CIN that increase with more advanced stages of presentation, suggesting that CIN may contribute to progression of this phenotype. In our series, none of the MSS proximal tumors had *BRAF* mutations and no distinct association was found with mucinous phenotype. Consequently, these phenotypes did not make a significant contribution to the MSS proximal cancers in the AA patients in our series, although other markers, such as CIMP and CIN, should be assessed. It is unclear why the phenotype described in these articles, although relatively infrequent, is basically not seen in our series, as we did not detect any *BRAF* V600E mutations in proximal MSS tumors in AAs. It is possible that AAs may have different *BRAF* mutations and our analysis restricted to V600E could limit this assessment. In any case, further molecular characterization in the described group will be essential to better understand this difference between AA and NHW colorectal cancers.

The much higher percentage of proximal colorectal cancers in AAs constitutes an added challenge for AAs, because these tumors are reportedly more likely to be missed by colonoscopies (10, 11) and interval cancers (colorectal cancers discovered at or before the next recommended screening/surveillance colonoscopy) have been repeatedly found to appear twice as often in the proximal colon (39).

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Could the additional proximal tumors in AAs be explained by factors such as toxic exposures, body habitus, obesity, or physical exercise? On the contrary, distal MSS tumors were more often diagnosed in obese patients and consumers of alcohol or tobacco, though our study probably was underpowered to prove this hypothesis. Younger AAs present with more distal tumors than proximal ones, which suggests that the increase of colorectal cancer in young AAs could be linked to environmental factors.

Our study had some limitations. Patients have been recruited within a limited geographical urban area with mostly modest income households; therefore, data may not be fully generalizable to other communities. The limited number of patients with data on such factors as toxic exposures, body habitus or exercise also reduces the possibility of drawing more firm conclusions on the differential effect of these factors.

In summary, our data strongly support the conclusion that the excess of proximal colorectal cancer in AAs consists of MSS tumors, commonly presenting lymphocytic infiltrate and less often associated with toxic exposures or a higher BMI. In addition, AAs are more often diagnosed with colorectal cancer at younger ages than NWHs. The clinical evidence suggests that the different mechanisms drive the younger ages of diagnosis and the proximal MSS colorectal cancers. Given the trend toward earlier cancer presentation, colorectal cancer screening approaches require further evaluation, especially in AAs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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