

Molecular Analysis of Colorectal Tumors within a Diverse Patient Cohort at a Single Institution

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Abstract

Purpose: African American colorectal cancer patients have worse survival outcomes than Caucasian patients. To determine whether differences exist in the molecular mechanisms driving colorectal cancer between African Americans and Caucasians, we characterized patient tumors from a single institution by assessing genetic alterations involved in colorectal cancer progression and response to treatment.

Experimental Design: We retrospectively examined 448 African Americans and Caucasians diagnosed with colorectal cancer at The University of Chicago Medical Center between 1992 and 2002. Microsatellite instability (MSI) status was determined by genotyping the *BAT25*, *BAT26*, *BAT40*, *D5S346*, and *BAX* loci. Mutations in *KRAS* codons 12 and 13 and *BRAF* codon 600 were identified by direct sequencing. MSI and detected mutations were correlated with clinicopathologic features.

Results: Overall, no difference existed in MSI or *BRAF* mutation frequencies between African Americans and Caucasians. However, African Americans with microsatellite stable (MSS)/MSI-low (MSI-L) tumors had a higher proportion of *KRAS* mutations than Caucasians (34% vs. 23%, $P = 0.048$) that was isolated to proximal colon cancers and primarily driven by mutations in codon 13. There was no racial difference in receipt of chemotherapy, but African Americans with MSS/MSI-L tumors had a 73% increased risk of death over Caucasians that could not be explained by known prognostic factors.

Conclusions: The significantly higher risk of death among African Americans with MSS/MSI-L tumors may be related to differences in the distribution of factors influencing response to standard therapies. These data underscore the need for further research into the molecular mechanisms driving colorectal cancer progression in underserved and understudied populations. *Clin Cancer Res*; 18(2); 350–9. ©2011 AACR.

Introduction

African Americans have the highest incidence and mortality rates of colorectal cancer in the United States. Unlike Caucasians, who display declining mortality rates, African American mortality rates have stabilized over the past 20

years for reasons not well understood (1). Although many factors are likely to play a role in the disparate survival outcomes between African American and Caucasian patients, whether tumor biology and genetics contribute to this observed health disparity have yet to be fully explored (2–7).

African Americans with colorectal cancer are typically diagnosed at a younger age than Caucasians (8, 9), with the largest mortality rate differences between African Americans and Caucasians being among younger patients and those diagnosed at early stages (2, 3). African Americans also have a higher incidence of proximal tumors than Caucasians for reasons that are unknown and understudied (8–10). Whereas survival rates do not vary significantly between African Americans and Caucasians with rectal adenocarcinomas, African Americans with colonic adenocarcinomas are more likely to die of their disease than Caucasians with colonic adenocarcinomas (6). These findings suggest that aggressive tumor phenotypes associated with poor prognostic outcomes are partly responsible for the disparities.

Colorectal cancer initiation and progression is driven by an accumulation of genetic mutations, leading to loss of genomic integrity through 3 identified patterns. The majority (80%–85%) of colorectal tumors display chromosomal instability, leading to defects in genes (e.g., *TP53*, *EGFR*, *KRAS*, *BRAF*, *APC*) that guard chromosomal integrity and

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Translational Relevance

Despite African Americans having the highest colorectal cancer incidence and mortality rates in the United States, there is a paucity of data driving policy to improve disparate population survival outcomes. To address the knowledge gap, we conducted molecular analysis of colorectal tumors from a cohort of African American and Caucasian patients to determine if differences existed in the distribution of genetic factors influencing colorectal cancer pathogenesis and progression. This study is relevant to the future practice of cancer medicine, because it is the most comprehensive analysis of an African American cohort treated at a single institution that incorporates genetic, molecular, pathologic, clinical, and treatment data to gain a better understanding of disparate survival outcomes. Our findings underscore the need for research into the science of cancer health disparities to examine the molecular mechanisms and pathways driving colorectal cancer progression in diverse patient populations.

regulate cell proliferation, differentiation, and apoptosis. A minority (15%) of tumors, having a better prognosis, progress through the microsatellite instability (MSI) pathway and is associated with inactivation of mismatch repair (MMR) genes. A third group (15%) of tumors, somewhat overlapping with MSI tumors, undergo aberrant methylation and display the CpG island methylator phenotype (CIMP; refs. 11, 12). The identification of these (epi)genetic alterations driving the development of colorectal tumors has been vital to understanding how tumors progress to aggressive phenotypes and in determining how these mechanisms can be targeted to improve patient outcomes.

Due to the paucity of data on colorectal cancer progression in African Americans, we conducted a comprehensive study examining the molecular pathogenesis of this disease in a cohort of patients at The University of Chicago Medical Center. We assessed MSI status and *KRAS* and *BRAF* mutations, as they are involved in disease initiation, progression, and treatment response (11–22). Although previously studied, these molecular pathways have not been characterized in African Americans, and their potential role in the aggressive tumor phenotypes and poor survival outcomes reported in the African American population has not been fully explored. To our knowledge, this is the first study to examine whether differential distribution of biological factors contributes to outcome differences among African Americans and Caucasians in a hospital-based cohort.

Materials and Methods

Patient and sample ascertainment

Our selection criteria for inclusion in this study were patients diagnosed with colorectal cancer between

1992 and 2002, who had invasive cancer, and who had accessible matching normal and tumor tissue blocks. A total of 1,539 patients, who underwent surgical resection at The University of Chicago Medical Center, were identified in The University of Chicago Tumor Registry. Of those patients, we were able to link 564 (37%) patients to The University of Chicago Department of Pathology database from which we abstracted patient pathology reports. Of these 564 patients, 448 (29%) patients had accessible normal and tumor tissue blocks for inclusion in this study (Supplementary Fig. 1). This study was given approval by The University of Chicago Institutional Review Board.

All samples were fixed in 10% formalin and embedded in paraffin. Inclusion criteria included tumor location in the colon or rectum and operative resection. Clinical information included age, sex, race/ethnicity, tumor size, grade, American Joint Committee on Cancer stage, receipt of 5-fluorouracil (5-FU) treatment, survival, and status at last follow-up. Samples were analyzed anonymously as lab researchers were blinded to clinical and pathologic data.

Pathologic assessment and DNA extraction

Representative areas of the lesions were carefully selected from hematoxylin and eosin stained sections. DNA was extracted from matched surrounding normal and tumor 50- μ tissue sections using the PUREGENE DNA Purification Kit (Gentra Systems) according to the manufacturer's instructions. The protocol was modified with the use of octane for deparaffinization.

MSI analysis

We designed fluorophore-labeled primers (Integrated DNA Technologies) targeting the following microsatellite loci: *BAT25* forward 5'-TCGCCTCCAAGAATCTAAGT-3', reverse 5'-TCTGCATTTAACTATGGCTC-3'; *BAT26* forward 5'-TGACTACTTTTGACTTCAGCC-3', reverse 5'-AACCAATCAACATTTTTAACCC-3'; *BAT40* forward 5'-ACAACCCTGCTTTTGTTCCT-3', reverse 5'-GTAGAGCAAGACCACCTTG-3'; *D5S346* forward 5'-ACTCACTCTAGTGATAAATCGGG-3', reverse 5'-AGCAGATAAGACAGTATTACTAGTT-3'; *BAX* forward 5'-TCCATCCAGGATCGAGCAGGGCGA-3', reverse 5'-CACTCGCTCAGCTTCTTGGTGGAC-3'. Loci were amplified by PCR and genotyped by The University of Chicago Cancer Research Center (UCCRC) DNA Sequencing Facility. Microsatellite marker stability was analyzed using GeneMapper software (Applied Biosystems). A locus was considered unstable if allelic addition/deletion occurred in the tumor DNA compared with patient matched normal DNA. Tumors were categorized as MSS if all markers were stable, MSI-low (MSI-L) if <30% were unstable, and MSI-high (MSI-H) if \geq 30% were unstable. MSI was evaluated independently by 2 researchers (B.E. Sylvester and J. Zhang), without knowledge of clinicopathologic data and assessed twice per patient. If a discrepancy occurred, that tumor was classified as undetermined and not included within the study

analysis. MSI status could not be determined for 57 (13%) tumors due to poor DNA quality (Supplementary Fig. S1).

KRAS and BRAF sequencing

We designed the following primers (Integrated DNA Technologies) targeting *KRAS* codons 12 and 13 and *BRAF* codon 600 for direct sequencing: *KRAS* forward 5'-TGTTCTAATATAGTCACATTTTCATT-3', reverse 5'-TCCTGCAC-CAGTAATATGC-3'; *BRAF* forward 5'-GCTTGCTCTGATAC-CAAAATGAG-3', reverse 5'-CCACAAAATGGATCCAGACA-3'. Regions were amplified by PCR, sequenced by the UCCRC DNA Sequencing Facility, then analyzed using Sequencher software (Gene Codes Corporation). Mutations identified were confirmed by independent reactions using reverse sequencing. If a discrepancy occurred, that tumor was classified as undetermined and not included within the study analysis. *KRAS* and *BRAF* mutation data were not available on 28 (6%) and 30 (7%) patients, respectively, due to insufficient DNA or failed sequencing reads due to poor DNA quality (Supplementary Fig. S1).

Statistical analysis

We compared demographic and clinical characteristics between African Americans and Caucasians using Fisher's exact tests for categorical variables such as anatomic site, *t* tests for continuous variables like age at diagnosis, and the Wilcoxon rank-sum test for ordinal variables such as tumor grade. We compared racial differences in MSI status, *KRAS* and *BRAF* mutation status, as well as mutation spectrum using Fisher's exact tests. Similarly, we explored demographic and clinical factors associated with *KRAS* and *BRAF* mutations using *t* tests, Fisher's exact tests, or Wilcoxon rank-sum tests as appropriate. We examined racial differences in demographic and clinical factors, as well as *KRAS* and *BRAF* mutations within the MSI strata. Survival curves were obtained by the Kaplan–Meier method, and differences in overall survival rates were analyzed with the log-rank test. Cox proportional hazard models were used to examine independent prognostic factors, including MSI status and *KRAS* and *BRAF* mutations, along with other clinicopathologic factors. To explore the reasons for racial disparity in overall survival, we first conducted a univariate analysis and estimated the unadjusted HR for African Americans compared with Caucasians. Then, we added demographic factors (age and sex), pathologic factors (grade, stage, and anatomic site), 5-FU treatment, and molecular markers (MSI status and *KRAS* and *BRAF* mutation status) to the Cox models in a successive manner. This model building was conducted separately for all patients, for MSI-H patients, and for MSS/MSI-L patients. We tested for an interaction between race and MSI status. The final Cox model included all of these factors, plus an interaction term of race and MSI status. Patients with missing data in a particular prognostic factor were excluded from the Cox model building. A *P* value < 0.05 was considered statistically significant. All statistical analyses were done using Stata 11.0 (StataCorp).

Results

Patient characteristics

For inclusion in this study, we identified 448 of 1,539 consecutive patients diagnosed with invasive colorectal cancer between 1992 and 2002 that had clinicopathologic data and accessible matching normal and tumor tissues (Supplementary Fig. S1). Of the 448 patients, 50% were African American and 46% were Caucasian, reflecting the general pattern of colorectal cancer patients seen at The University of Chicago Medical Center. The remaining patients were of Asian American, Hispanic American, and unknown ancestry. We compared the clinicopathologic features of African Americans and Caucasians and found they were very similar, except African Americans in this hospital-based cohort were slightly older (69 vs. 66 years, *P* = 0.008). Of note, there was no difference in the receipt of chemotherapy by race within this cohort (Supplementary Table S1).

Molecular characterization of tumors

We evaluated the MSI status of all 448 cases. Of 391 tumors that could be scored (Supplementary Fig. S1), 73% were microsatellite stable (MSS), 13% were MSI-low (MSI-L), and 14% were MSI-high (MSI-H). We found no difference in the frequency of MSI tumors between African Americans and Caucasians. MSS and MSI-L cases were combined for analysis, as these cases are clinically and pathologically similar (12, 23, 24). Clinicopathologic data was available for 95% of cases with MSI data as shown in Table 1. In patients with MSS/MSI-L tumors, African Americans were older and more likely to be female than Caucasians. There were trends toward significance of Caucasians with MSS/MSI-L tumors having a higher proportion of poorly differentiated tumors and African Americans with MSS/MSI-L tumors presenting more frequently with proximal colon tumors.

We successfully tested for *KRAS* and *BRAF* mutations on 94% and 93% of cases, respectively (Supplementary Fig. S1). We focused on *KRAS* codons 12 and 13 mutations and the *BRAF* V600E mutation, as they respectively account for 90% of all *KRAS* mutations and 80% of all *BRAF* mutations in colorectal cancers (14, 25, 26). The overall frequency of *KRAS* mutations was 27% (115/420). Codon 12 mutations were more common, accounting for 77% (89/115) of all *KRAS* mutations identified. Twenty-four (21%) patients had codon 13 mutations, and 2 patients had mutations in both codons 12 and 13. The *BRAF* mutation frequency was 8% (32/418). Only 1 patient had mutations in both *KRAS* and *BRAF*, indicating comutation of *BRAF* and *KRAS* was uncommon (*P* = 0.001).

Table 2 presents the correlates for *KRAS* or *BRAF* mutation status. Both *KRAS* (*P* = 0.048) and *BRAF* (*P* = 0.003) mutations were associated with proximal colon tumors. Patients with a *BRAF* mutation were older compared with patients without the mutation (73 years vs. 67 years, *P* = 0.02). In addition, the *BRAF* mutation was more common in females (*P* = 0.02), poorly differentiated

Table 1. Patient clinicopathologic features stratified by MSI status and race

Factors	MSS/MSI-L			MSI-H		
	African Americans	Caucasians	P	African Americans	Caucasians	P
Overall	170 (85%)	150 (87%)	0.37	29 (15%)	22 (13%)	0.37
Age at diagnosis, mean ± SD	69.1 ± 12.5	65.4 ± 13.4	0.01	66.0 ± 14.6	67.1 ± 17.3	0.81
Sex						
Female	105 (62%)	72 (48%)	0.02	17 (59%)	13 (59%)	1.00
Male	65 (38%)	78 (52%)		12 (41%)	9 (41%)	
Grade						
Well differentiated	37 (22%)	25 (17%)	0.07	2 (7%)	3 (14%)	0.89
Moderately differentiated	112 (68%)	95 (66%)		20 (69%)	12 (54%)	
Poorly differentiated	16 (10%)	24 (17%)		7 (24%)	7 (32%)	
AJCC stage						
I	30 (18%)	29 (19%)	0.84	5 (17%)	6 (29%)	0.29
II	50 (30%)	41 (28%)		12 (42%)	8 (38%)	
III	55 (33%)	45 (30%)		9 (31%)	7 (33%)	
IV	33 (20%)	34 (23%)		3 (10%)	0 (0%)	
Anatomic site						
Proximal colon	94 (55%)	68 (45%)	0.06 ^a	22 (76%)	17 (77%)	0.67 ^a
Distal colon	59 (35%)	61 (41%)		6 (21%)	2 (9%)	
Rectum	4 (2%)	10 (7%)		1 (3%)	0 (0%)	
Colon, unspecified	13 (8%)	11 (7%)		0 (0%)	3 (14%)	
5-FU treatment	44 (28%)	49 (34%)	0.26	8 (30%)	8 (38%)	0.56
KRAS mutation	57 (34%)	35 (23%)	0.048	7 (24%)	5 (23%)	1.00
BRAF mutation	6 (4%)	7 (5%)	0.78	11 (39%)	6 (27%)	0.55

Abbreviations: AJCC, American Joint Committee on Cancer; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable.

^aP value was calculated after excluding patients with unspecified anatomic location of colon cancers.

tumors ($P = 0.005$), and stage 2 and 3 cancers ($P = 0.01$). The *BRAF* mutation was also highly associated with MSI-H tumors ($P < 0.001$).

KRAS mutation frequency and spectrum by race

Among patients with MSS/MSI-L tumors, African Americans had a higher frequency of *KRAS* mutations compared with Caucasians (34% vs. 23%, $P = 0.048$), especially in codon 13 (Table 1, Fig. 1A). The spectrum of mutations also varied between the 2 populations (Fig. 1B and C). African Americans were more likely to have the c.35G>A (p.G12D) mutation in codon 12, while both the c.35G>A (p.G12D) and c.35G>T (p.G12V) mutations were the most common in Caucasians ($P = 0.02$). Within codon 13, African Americans outnumbered Caucasians with the c.38G>A (p.G13D) mutation, and this was nearly statistically significant ($P = 0.058$). Interestingly, the difference in *KRAS* mutation frequency between African Americans and Caucasians occurred in MSS/MSI-L tumors within the proximal colon (44% vs. 21%, $P = 0.002$); whereas in the distal colon (22% vs. 21%, $P = 1.0$), rectum (23% vs. 46%, $P = 0.39$), and colon [(unspecified) 0% vs. 30%, $P = 0.51$], the mutation frequency was similar (Fig. 1D).

Prognostic factors for overall survival and racial disparity

Survival outcomes were available for 446 (99%) patients. After a median follow-up of 8.8 years, the cohort median survival time was 6.5 years (95% CI: 5.4–9.1). To examine the reasons for the observed racial differences in overall survival, we developed Cox models comparing African Americans to Caucasians, adjusting for clinical and molecular variables in a serial pattern among all patients and separately according to MSI status (Table 3). In the univariate analysis (Model A), African Americans had a 14% increased risk of death over Caucasians that was not statistically significant. After adjustment for age, sex, grade, stage, and anatomic site (Model C), African Americans had a 35% increased risk of death over Caucasians that was statistically significant. Survival differences between African Americans and Caucasians remained statistically significant even after adjustment for receipt of 5-FU, MSI status, and *KRAS* and *BRAF* mutations (Model D, Model E, Model F, and Full Model, respectively). In the univariate analysis, African Americans had worse survival than Caucasians among patients with MSS/MSI-L tumors and with MSI-H tumors (Fig. 2A and B). Following adjustment for clinicopathologic and molecular factors (Full Model), African Americans had

Table 2. Clinicopathologic characteristics of patients with *KRAS* or *BRAF* mutations

Factors	Wild-type <i>KRAS</i>	Mutant <i>KRAS</i>	<i>P</i>	Wild-type <i>BRAF</i>	Mutant <i>BRAF</i>	<i>P</i>
Age at diagnosis, mean ± SD	66.7 ± 13.3	69.1 ± 12.4	0.10	66.9 ± 13.2	72.5 ± 11.3	0.02
Sex						
Female	177 (58%)	62 (54%)	0.44	214 (56%)	25 (78%)	0.02
Male	127 (42%)	53 (46%)		171 (44%)	7 (22%)	
Race						
African American	146 (51%)	65 (59%)	0.15	192 (53%)	18 (56%)	0.72
Caucasian	143 (49%)	45 (41%)		173 (47%)	14 (44%)	
Grade						
Well differentiated	52 (18%)	24 (22%)	0.14	75 (20%)	1 (3%)	0.005
Moderately differentiated	193 (65%)	72 (66%)		242 (65%)	22 (69%)	
Poorly differentiated	51 (17%)	13 (12%)		55 (15%)	9 (28%)	
AJCC stage						
I	60 (20%)	21 (19%)	0.30	79 (21%)	2 (6%)	0.01
II	89 (30%)	31 (27%)		105 (28%)	15 (47%)	
III	102 (34%)	35 (31%)		122 (32%)	13 (41%)	
IV	49 (16%)	26 (23%)		73 (19%)	2 (6%)	
Anatomic site						
Proximal colon	153 (50%)	71 (62%)	0.048 ^a	196 (51%)	26 (81%)	0.003 ^a
Distal colon	115 (38%)	29 (25%)		140 (36%)	4 (13%)	
Rectum	15 (5%)	5 (4%)		20 (5%)	0 (0%)	
Colon, unspecified	21 (7%)	10 (9%)		29 (8%)	2 (6%)	
5-FU treatment						
No	187 (66%)	80 (72%)	0.23	247 (68%)	20 (62%)	0.56
Yes	98 (34%)	31 (28%)		116 (32%)	12 (38%)	
MSI status						
MSS/MSI-L	240 (86%)	96 (88%)	0.62	323 (90%)	13 (43%)	<0.001
MSI-H	40 (14%)	13 (12%)		35 (10%)	17 (57%)	

Abbreviations: AJCC, American Joint Committee on Cancer; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable.

^a*P* value was calculated after excluding patients with unspecified anatomic location of colon cancers.

a 73% increased risk of death over Caucasians among patients with MSS/MSI-L tumors, but there was no survival difference between African Americans and Caucasians with MSI-H tumors (*P* for interaction = 0.10).

Table 4 presents all prognostic factors in the Full Model. As expected, high grade and advanced stage were associated with poor overall survival. Age at diagnosis was significantly associated with overall survival specifically in a piecewise manner: before age 65, the risk of death increased 27% per 10-year age increment; after age 65, the risk of death significantly increased 40% per 10-year age increment. Encouragingly, patients who received 5-FU-based treatment had a 45% reduced risk of dying after controlling for all other prognostic factors. There was a trend toward significance that the *BRAF* mutation was associated with worse survival (also see Fig. 2C and D). However, the presence of a *KRAS* mutation did not correlate with overall survival (also see Fig. 2E and F).

Discussion

We conducted a comprehensive analysis characterizing the colorectal tumors of self-reported African American and

Caucasian patients in a hospital-based study to determine if biological factors contributed to reported poorer survival outcomes in African Americans. Overall, we found that African Americans had a 59% increased risk of death compared with Caucasians, following adjustment for demographic factors. This is similar to what is reported in U.S. population studies (1). African Americans and Caucasians had similar demographics in this study, except age at diagnosis, in which African Americans were slightly older. The older age was likely caused by the referral pattern at The University of Chicago Medical Center, which has a tertiary cancer center that is influenced by insurance status and a higher referral rate of young Caucasians from regional hospitals. However, adjustment for known prognostic factors in the multivariate analysis, including age, did not explain the poorer survival of African Americans within this study.

To examine what factors may have contributed to the poorer prognosis of these patients, we examined overall survival stratified by MSI status, because MSI-H tumors represent a clinically and pathologically distinct disease from MSS and MSI-L tumors. Prior studies show that MSI-H tumors have a pathologic, molecular, and clinical profile

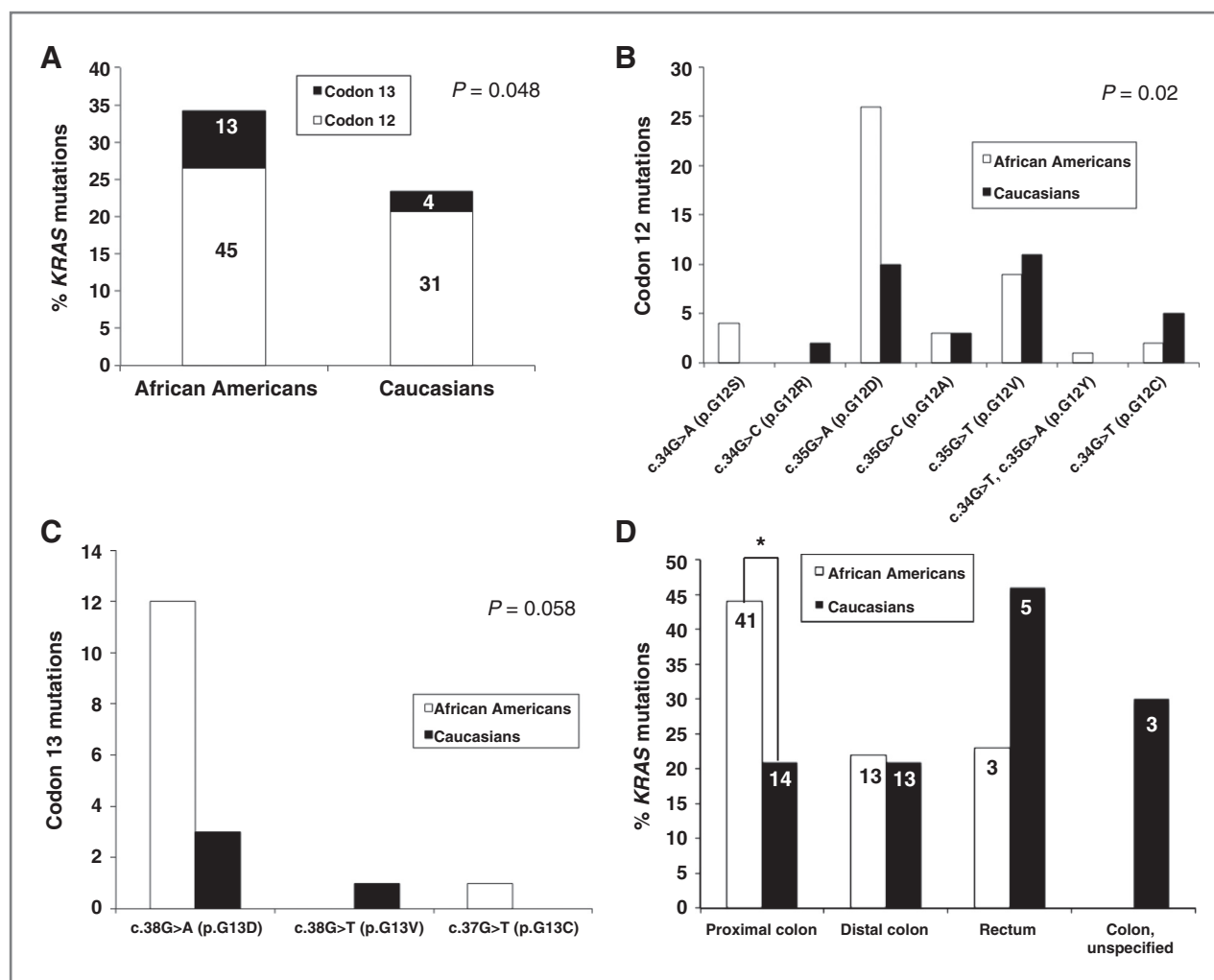


Figure 1. KRAS codons 12 and 13 mutation frequencies and spectra of African American and Caucasian patients with MSS/MSI-L tumors. A, overall frequency of KRAS mutations by codon. Numbers on the column charts represent the raw number of KRAS-mutated cases for each population by codon. B, spectrum of mutations in KRAS codon 12. C, spectrum of mutations in KRAS codon 13. D, overall frequency of KRAS mutations by anatomic site. Numbers on the column charts represent the raw number of KRAS-mutated cases for each population by anatomic site. *, $P = 0.002$. MSS, microsatellite stable; MSI-L, microsatellite instability-low.

distinct from other colorectal cancer subtypes. These tumors tend to be proximally located, of a high histologic grade, and interestingly have an improved prognosis over MSS/MSI-L tumors, which may be due to MSI-H tumors prominent infiltration of lymphocytes, tendency to be of low pathologic stage, and lower propensity to metastasize (27). As with previous studies (12, 27, 28), our analysis showed that patients with MSI-H tumors had significantly better overall survival than patients with MSS/MSI-L tumors, and this was irrespective of race. We did not detect a difference in the frequency of MSI tumors or in the pattern of MMR protein expression (data not shown) between African Americans and Caucasians, contradicting reported differences in the loss of MMR pathway function between these 2 groups (20, 29–31). It is important to note that our cohort was older in age, and extensive analysis of MSI-H cases was limited by statistical power due to the low number of cases.

Next, we examined KRAS and BRAF mutations to determine if these genetic alterations contributed to the disparate survival outcomes between African Americans and Caucasians, as defects in the mitogen-activated protein kinase (MAPK) pathway affect colorectal cancer progression, response to therapy, and survival outcome (11, 14, 16). We found that 27% of patients had KRAS codons 12 or 13 mutations and 8% of patients had the BRAF V600E mutation. The BRAF frequency was similar to previously reported studies (10%–15%; refs. 15, 18, 32, 33). The KRAS frequency was lower than the 32% to 40% range reported in other studies (22, 34, 35). The overall KRAS mutation spectrum resembled that of previous studies, with c.35G>A (p.G12D) and c.35G>T (p.G12V) being the most prevalent codon 12 mutations and c.38G>A (p.G13D) being the most prevalent codon 13 mutation (19, 21, 22, 36). Both KRAS and BRAF

Table 3. Cox models: risk of death for African American patients relative to Caucasian patients

Models	All patients		MSS/MSI-L		MSI-H	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Model A: unadjusted	1.14 (0.89–1.46)	0.30	1.24 (0.93–1.65)	0.14	1.05 (0.43–2.57)	0.92
Model B: adjusted for age and sex	1.07 (0.83–1.37)	0.61	1.16 (0.87–1.54)	0.31	1.05 (0.43–2.56)	0.92
Model C: Model B + adjustment for grade, stage, and anatomic site	1.35 (1.03–1.77)	0.03	1.65 (1.21–2.25)	0.002	0.91 (0.36–2.25)	0.83
Model D: Model C + adjustment for 5-FU treatment	1.32 (0.99–1.75)	0.06	1.69 (1.22–2.34)	0.002	0.75 (0.30–1.88)	0.54
Model E: Model D + adjustment for MSI status	1.56 (1.14–2.12)	0.005	—	—	—	—
Model F: Model E + adjustment for <i>KRAS</i> mutation status	1.58 (1.16–2.16)	0.004	1.72 (1.24–2.39)	0.001	0.75 (0.30–1.90)	0.55
Full Model: Model F + adjustment for <i>BRAF</i> mutation status	1.59 (1.16–2.18)	0.004	1.73 (1.24–2.40)	0.001	0.75 (0.29–1.89)	0.54

Abbreviations: MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable.

mutations associated with proximal colon tumors, and *BRAF* mutations were more common in older patients, females, poorly differentiated tumors, stage II and III cancers, and MSI-H tumors. These observations are also aligned with previous studies (15, 36). No differences were identified in *BRAF* mutation frequencies between African Americans and Caucasians; however, our analysis was limited by the low overall representation of mutant *BRAF* cases. We did identify a significantly higher frequency (11% difference) of *KRAS* mutations in African Americans than in Caucasians with MSS/MSI-L tumors. This observation was particularly interesting considering the most significant difference in mutation frequency was found in codon 13 and not in the predominantly mutated codon 12. The overall lower *KRAS* mutation frequency within this study may be due to the diversity of cases and the referral pattern at The University of Chicago Medical Center, and replication of our findings is warranted in a larger cohort.

We saw a trend among our patients in which a *BRAF* mutation was associated with poorer overall survival, and this data is in agreement with previously reported studies of this mutation serving as a poor prognostic indicator (15, 32, 36–38). In the univariate analysis, *KRAS* mutations had no prognostic value, which is in agreement with previous studies (34, 36, 39). Furthermore, we included *KRAS* mutation status in the multivariate analysis to determine its influence on disparate survival between African Americans and Caucasians, but it had no impact on survival differences. However, we did determine that the racial difference in the frequency of *KRAS* mutations among MSS/MSI-L cases was confined to tumors in the proximal colon. In proximal colon cancers, 44% of African Americans with MSS/MSI-L tumors had *KRAS* mutations compared with 21% in Caucasians with MSS/MSI-L tumors. No difference existed in the frequency of *KRAS* mutations among distal,

rectal, or colon (unspecified) cancers in patients with MSS/MSI-L tumors. Tumors of the proximal colon have a different pathology than tumors of the distal colon and rectum, which suggest differences in causative agents of disease initiation and progression. Our data, thus, point to possible etiologic differences behind disease initiation in the proximal colon between African Americans and Caucasians. Many studies have reported the effect of diet on tumor development in the colon and rectum, documenting the protective effects of insoluble fibers and phenolic substances (found in fruits, vegetables, wine, and many other foods) on colorectal carcinogenesis, along with the increased risk of adenomas, hyperplastic polyps, and colorectal cancer associated with the consumption of meat-derived mutagens found in red and processed meats cooked at high temperatures (40–44). *KRAS* mutations are particularly characteristic of the type of alkylative damage that can be caused by carcinogens such as *N*-nitroso compounds (42). Dietary and lifestyle patterns can vary among racial/ethnic populations, leading to varied exposures that may impact the risk and type of disease developed. Further research is needed to determine the origins of racial differences in the spectrum and frequency of *KRAS* mutations and to determine how environmental exposures and (epi)genetics influence tumor pathogenesis in the proximal colon versus the distal colon and rectum.

One mechanism we did not explore in this analysis, due to limited tissue availability, was the extent of widespread promoter methylation and the proportion of cases that would be classified with high or low levels of CIMP. The CIMP-high phenotype has been reported in approximately 15% of colorectal cancer cases in population-based studies (45, 46) and highly correlates with the *BRAF* V600E mutation, MSI-H status, proximally located tumors, and older age (45–47). The CIMP-low phenotype correlates with a higher proportion of *KRAS* mutations

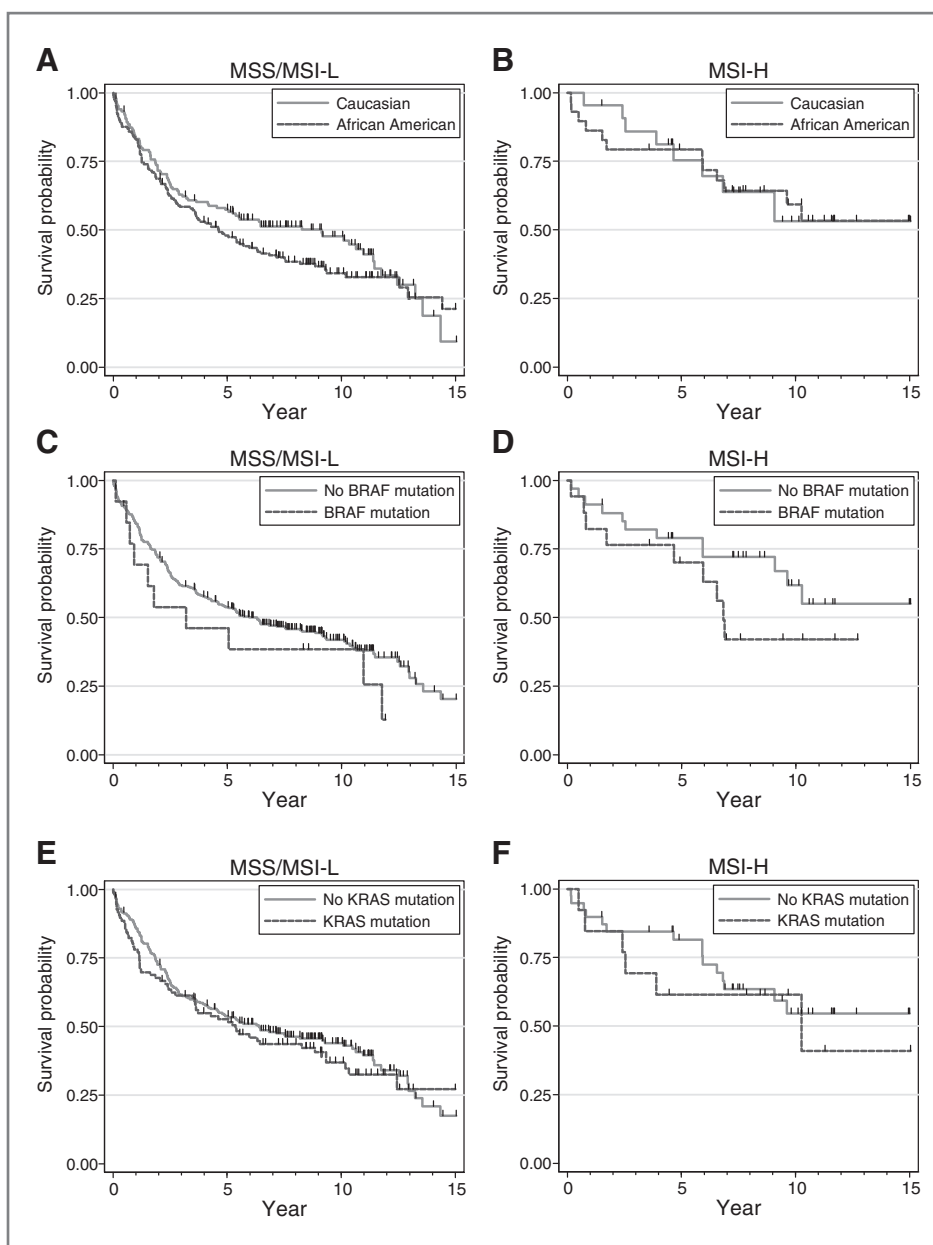


Figure 2. Kaplan-Meier curves displaying patient overall survival, stratified by MSI status. A, overall survival by race in patients with MSS/MSI-L tumors. B, overall survival by race in patients with MSI-H tumors. C, overall survival of patients with or without a *BRAF* mutation in MSS/MSI-L cases. D, overall survival of patients with or without a *BRAF* mutation in MSI-H cases. E, overall survival of patients with or without a *KRAS* mutation in MSS/MSI-L cases. F, overall survival of patients with or without a *KRAS* mutation in MSI-H cases. MSS, microsatellite stable; MSI-L, microsatellite instability-low; MSI-H, microsatellite instability-high.

compared with CIMP-high tumors, and patients with MSS tumors that are either CIMP-high or CIMP-low have shorter 5-year survival rates than patients with tumors containing no level of CIMP (46). As increasing levels of CIMP are linked to poorer patient survival, especially in MSS cases, this phenotype deserves evaluation in diverse populations to determine whether its frequency varies among populations and whether it impacts disparate survival rates.

Limitations of this study were the retrospective nature and the overrepresentation of older patients seen at a single institution, thereby preventing the analysis of young onset colorectal cancer cases. In addition, the sample size of some subgroups, like MSI-H and *BRAF*

mutant cases, was small; hence, replication in large patient cohorts is needed. We also did not assess the influence of comorbidities. For future studies, additional data integrating lifestyle and environmental risk factors, as well as factors affecting receipt and tolerance of treatment in diverse populations, must be considered to determine the complex underlying factors that interact with tumor biology to influence poor outcomes (48).

In the first comprehensive study of its kind that incorporates clinical, pathologic, genetic, and molecular data, we found that African American colorectal cancer patients had a significantly higher risk of death than Caucasian patients that was limited to MSS/MSI-L cases and could not be explained by known prognostic factors. This study advances

Table 4. Cox model: multivariate analysis of prognostic factors for overall survival

Factors	HR (95% CI)	P
Age at diagnosis (per 10 years)		
<65 year stratum	1.27 (0.96–1.70)	<0.0001
≥65 year stratum	1.40 (1.13–1.74)	
Male vs. Female	1.14 (0.84–1.55)	0.39
Grade		
Well differentiated	1.0 (ref)	0.02
Moderately differentiated	1.42 (0.91–2.20)	
Poorly differentiated	1.97 (1.12–3.46)	
AJCC stage		
I	1.0 (ref)	<0.0001
II	2.13 (1.29–3.51)	
III	2.74 (1.59–4.73)	
IV	20.23 (11.57–35.39)	
Anatomic site		
Distal colon	1.0 (ref)	0.84
Proximal colon	1.04 (0.75–1.44)	
Rectum	1.23 (0.61–2.51)	
5-FU treatment	0.55 (0.37–0.81)	0.002
African American vs. Caucasian		
In MSS/MSI-L	1.73 (1.24–2.40)	0.001
In MSI-H	0.75 (0.29–1.89)	0.54
<i>BRAF</i> mutation	1.59 (0.88–2.86)	0.13
<i>KRAS</i> mutation	0.93 (0.67–1.31)	0.69

Abbreviations: AJCC, American Joint Committee on Cancer; ref, reference.

the science of cancer health disparities and opens up the field for further inquiry into whether observed survival differences may be related to differences in the distribution of risk factors that determine response to standard therapies. This study underscores the need for extensive characterization of cancer genomes from diverse patient populations that can lead to improved approaches to the treatment and prevention of cancer; only then can we reduce health disparities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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