OXFORD

JNCI J Natl Cancer Inst (2016) 108(12): djw164

doi: 10.1093/jnci/djw164
First published online August 31, 2016
Brief Communication

BRIEF COMMUNICATION

Adverse Clinical Outcome Associated With Mutations That Typify African American Colorectal Cancers

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Abstract

African Americans have the highest incidence and mortality from colorectal cancer (CRC) of any US racial group. We recently described a panel of 15 genes that are statistically significantly more likely to be mutated in CRCs from African Americans than in Caucasians (AA-CRC genes). The current study investigated the outcomes associated with these mutations in African American CRCs (AA-CRCs). In a cohort of 66 patients with stage I-III CRCs, eight of 27 CRCs with AA-CRC gene mutations (Mut+) developed metastatic disease vs only four of 39 mutation-negative (Mut-) cases ($P = .03$, Cox regression model with two-sided Wald test). Moreover, among stage III cases ($n = 33$), Mut+ cancers were nearly three times more likely to relapse as Mut- cases (7 of 15 Mut+ vs 3 of 18 Mut-; $P = .03$, Cox regression model with two-sided Wald test). AA-CRC mutations may thus define a high-risk subset of CRCs that contributes to the overall disparity in CRC outcomes observed in African Americans.

African Americans are more likely to be diagnosed with, and to die of, colorectal cancer (CRC) than any other ethnic or racial group, with 25% higher incidence and 50% higher mortality than Caucasians (1–5). African Americans, moreover, experience worse stage-specific survival than Caucasians. Mortality rates for Caucasian men with CRC have declined by up to 39% but have increased by 28% for African American men since 1960 (6,7). The contributions of biological differences vs socio-economic disparities to these striking disparities are largely unknown. Our team recently completed the first genome-wide analysis of the mutational landscape of 102 microsatellite-stable CRCs (93 primaries; 8 liver and 1 lung metastases) arising in African Americans (8). We identified recurrent mutations in 20 new candidate CRC driver genes in these African American tumors, finding that this panel of genes was mutated twice as often in colon cancers from African Americans than from Caucasians. This effect was driven by 15 of these genes that, together, were 3.3-fold more commonly mutated in African American than in Caucasian CRCs. Forty-one percent of African American CRCs had somatic mutations in at least one of the 15 genes. Furthermore, 14% of African American CRCs harbored exclusive mutations in four genes (*EPHA6, FLCN*, *HTRIF*, and *WASH1*).

To ascertain whether the 15-gene AA-CRC mutational panel might contribute to worse outcomes in African American colorectal cancer patients, we performed a retrospective review of 102 African American patients from Case Medical Center under a Case Medical Center institutional review board–approved discarded tissue protocol. Of these previously studied cases, 42 cancers were positive for AA-CRC gene mutations (Mut+) and 60 were negative (Mut-). Patient outcomes, obtained by chart reviews, were available for 93 patients (40 Mut+ and 53 Mut-). Thirty-nine patients (21 Mut+ and 18 Mut-) presented with stage IV disease or developed metastases after surgery of a primary cancer. Of these, 23 patients (11 Mut+ and 12 Mut-) presented with stage IV disease, and 16 non–stage IV patients (10 Mut+ and 6 Mut-) had disease recurrence. Fifty-four patients

Received: January 25, 2016; **Revised:** April 24, 2016; **Accepted:** May 25, 2016

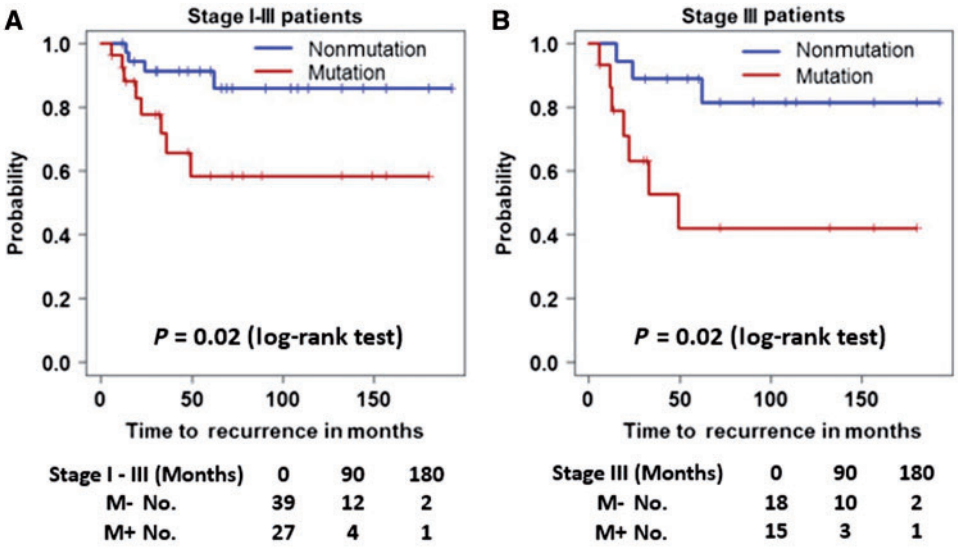

Figure 1. Kaplan-Meier analysis of disease-free survival in colorectal cancer (CRC) cases with (Mutation) or without (Nonmutation) in the 15-gene African American CRC (AA-CRC) gene panel. **A)** Analysis of stage II-III patients is shown demonstrating decreased disease-free survival in African American colon cancer patients with the AA-CRC gene mutation ($P = .02$). **B)** Analysis of stage III patients is shown demonstrating decreased disease-free survival in African American colon cancer patients with the AA-CRC gene mutation ($P = .02$). Stage III cases are a subset of the stage I-III cases of **(A)**. *P* values were calculated using two-sided log-rank tests. **Tables below plots** indicate patients at risk at timepoints 0, 90, and 180 months.

with stage I-III disease (19 Mut+ and 35 Mut-) had no evidence of recurrence, with a median follow-up of 60 months (range = 6–192 months).

Our initial study sequenced stage IV/ liver metastasis cases as a discovery set, creating a potential ascertainment bias by enrichment for Mut+ advanced-stage cases (8). To perform an unbiased assessment of the effect of AA-CRC mutations, we examined the relationship of mutational status and outcomes in stage I-III and stage III–only patients. Difference in time to disease recurrence between Mut+ and Mut- patients was assessed by Cox regression model with Wald test *P* values and Kaplan-Meier plots with log-rank test *P* values. All statistical tests were two-sided, and a *P* value of less than .05 was considered statistically significant.

Sixty-six patients with stage I-III CRCs (27 Mut+ and 39 Mut-) had been followed for disease recurrence without regard to their mutational status, with a median follow-up time of 57 months (range = 6–193 months). In these patients, Mut+ CRCs were associated with a statistically significantly increased relapse rate hazard ratio (HR) of 3.92 (95% confidence interval [CI] = 1.18 to 13.09, $P = .03$) and shorter disease-free survival (log-rank test $P = .02$) (Figure 1A), with eight of 27 AA-CRC Mut+ cases relapsing compared with only four of 39 AA CRC Mut- cases.

Thirty-three of these 66 patients were stage III CRCs (15 Mut+ and 18 Mut-), all of whom had been followed for disease recurrence without regard to their mutational status, with a median follow-up time of 60 months (range = 6–193 months). In this subset of stage III CRCs, the presence of AA-CRC mutations was again statistically significantly associated with adverse outcome. Specifically, Mut+ stage III cases showed a statistically significantly higher relapse rate hazard ratio of 4.53 (95% CI = 1.16 to 17.72, $P = .03$), with a 60-month shortened median disease-free survival (95% CI = 30.5 to 94.1, $P = .02$ by quantile regression) and a statistically significantly reduced actuarial disease-free survival (log-rank test $P = .02$) (Figure 1B). Overall, seven of 15 Mut+ stage III cases relapsed vs only three of 18 Mut- cases.

Among the 15 AA-CRC genes, EPHA6 was targeted at the highest frequency for somatic mutation, with a 6% mutation frequency detected in African American CRCs and with no mutations detected among Caucasian cancers. Clinical follow-up data was available in five of six patients whose cancers harbored EPHA6 mutations. Out of these five patients, four had poor outcomes: two patients presented with liver metastases, one stage II patient relapsed with liver metastasis within three years, and one stage III patient relapsed within six months with multiple bone metastases. The fifth patient had stage II disease, with over five years of disease-free survival. The single patient lost to follow-up had metastatic cancer in 19 regional lymph nodes at colectomy.

Pathology slides were available from primary tumors for 87 cases. Thirty-seven cancers were positive for one or more AA-CRC gene mutations (Mut+), and 50 were mutation negative (Mut-). These cases were reviewed by two GI pathologists (YSP and JW), who were blinded to the mutation status of each cancer and to patient outcomes. Pathology parameters assessed were: tumor classification, tumor grade, presence of angiolymphatic or perineural invasion, infiltrative growth pattern, and a prominent peritumoral lymphocytic infiltrate—features known to stratify with risk of CRC recurrence (9–11). A greater than 20% infiltrative growth pattern was classified as infiltrative overall (Supplementary Figure 1, available online).

Among the pathological parameters assessed, the presence of an infiltrative growth pattern in AA-CRC Mut+ CRCs had a nominal *P* value of .02 (two-sided Fisher's exact test) (Table 1); that is, this size cohort falls short of statistical significance when corrected for multiple testing using false discovery rate methodology. (12) No differences in patient ages were noted between the two groups (Supplementary Table 1, available online).

The study's principal limitation is that all patients derive from one tertiary care institution. Although the association of this mutational panel with ethnicity and clinical outcome is striking, it is certainly possible that these mutations are a marker of a confounding exposure or other epidemiologic difference that is unique to this community. Thus, further validation studies with patient cohorts from various regions of the country are warranted.

Table 1. Summary clinical and pathology characteristics from 86 African American colon cancer patients

Summary characteristics	All cases (n = 86)	AA-CRC Mut + (n = 35)	AA-CRC Mut - (n = 51)
Mean age (range), y	66 (29–92)	66 (29–86)	65 (33–92)
TNM stage, No.			
I-II	41	15	26
III	36	16	20
IV	23	11	12
Unknown	2	0	2
Primary tumor site, No.			
Right	66	29	37
Left	30	11	19
Rectum	4	1	3
Unknown	2	1	1
Primary tumor grade, No.			
Low	78	29	49
High	8	6	2
Infiltrating pattern, No.			
Yes	52	26	26
No	33*	8	27
Angiolymphatic invasion, No.			
Yes	22	12	10
No	64	23	41
Perineural invasion, No.			
Yes	20	9	11
No	66	26	41
Peritumoral lymphocytic infiltrate*, No.			
Yes	11	6	5
No	75	29	46
Tumor type, No.			
Intestinal	66	30	36
Mucinous differentiation	19	4	15
Other	1	1	0

*One specimen not interpretable.

In summary, we have identified an association of poor clinical outcome in African American patients whose CRCs contain mutations in a 15-gene mutational panel (AA-CRC genes) predominantly found in African American CRCs. Our study suggests that AA-CRC gene mutations may define a high-risk subset of colon cancers that contributes to the overall observed disparity in colon cancer outcomes seen in African Americans. It will be of interest with larger studies to determine if the AA-CRC mutation panel may also define high-risk cancers that arise, albeit at lower frequency, in other ethnic groups. Moreover, further studies examining the AA-CRC gene mutational panel in additional cohorts of African American colon cancer patients, as well as in model systems, will also be clearly warranted to help distinguish the role of these mutations as markers vs mediators of adverse outcome.

Funding

This project was supported by National Institutes of Health awards: Case GI SPORE P50 CA150964 (to SDM), KO8 CA148980 (to KG), R01-CA204549 (to KG), R21 CA149349 (to JW), and P30 CA043703 and the V Foundation for Cancer Research Award T2015-007.

Notes

The funders had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

We gratefully acknowledge the work of Beverly McGloin and Susan Lewis in organizing the biospecimen and clinical data repository central to this study.

Paper presented at meeting: Novel Mutations in African American Colon Cancers Define a Distinct Clinicopathological Entity Linked to Poor Outcome. Joseph Willis, Li Li, Martina Veigl et al. United States and Canadian Academy of Pathology, 2016.

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