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Associations Between Colorectal Cancer Molecular Markers and Pathways With Clinicopathologic Features in Older Women

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Abstract

BACKGROUND & AIMS—Colorectal tumors have a large degree of molecular heterogeneity. Three integrated pathways of carcinogenesis (ie, traditional, alternate, and serrated) have been proposed, based on specific combinations of microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and mutations in *BRAF* and *KRAS*. We used resources from the population-based Iowa Women's Health Study (n = 41,836) to associate markers of colorectal tumors, integrated pathways, and clinical and pathology characteristics, including survival times.

METHODS—We assessed archived specimens from 732 incident colorectal tumors and characterized them as microsatellite stable (MSS), MSI high or MSI low, CIMP high or CIMP low, CIMP negative, and positive or negative for *BRAF* and/or *KRAS* mutations. Informative marker data were collected from 563 tumors (77%), which were assigned to the following

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Conflicts of interest This author discloses the following: Paul J. Limburg served as a consultant for Genomic Health, Inc. from August 12, 2008 to April 19, 2010. Mayo Clinic has licensed Dr. Limburg's intellectual property to Exact Sciences, and he and Mayo Clinic have contractual rights to receive royalties through this agreement. The intellectual property delivered through this prior relationship had no direct bearing on the current study, and Exact Sciences was not involved with the current study in any way. The remaining authors disclose no conflicts.

integrated pathways: traditional (MSS, CIMP negative, *BRAF* mutation negative, and *KRAS* mutation negative; n = 170), alternate (MSS, CIMP low, *BRAF* mutation negative, and *KRAS* mutation positive; n = 58), serrated (any MSI, CIMP high, *BRAF* mutation positive, and *KRAS* mutation negative; n = 142), or unassigned (n = 193). Multivariable-adjusted Cox proportional hazards regression models were used to assess the associations of interest.

RESULTS—Patients' mean age ($P = .03$) and tumors' anatomic subsite ($P = .0001$) and grade ($P = .0001$) were significantly associated with integrated pathway assignment. Colorectal cancer (CRC) mortality was not associated with the traditional, alternate, or serrated pathways, but was associated with a subset of pathway-unassigned tumors (MSS or MSI low, CIMP negative, *BRAF* mutation negative, and *KRAS* mutation positive) (n = 96 cases; relative risk = 1.76; 95% confidence interval, 1.07–2.89, compared with the traditional pathway).

CONCLUSIONS—We identified clinical and pathology features associated with molecularly defined CRC subtypes. However, additional studies are needed to determine how these features might influence prognosis.

Keywords

Molecular Epidemiology; Colon Cancer; Prognostic Factor; Integrated Pathways

Based on recent global estimates, colorectal cancer (CRC) is the third most common malignancy worldwide.¹ Although often viewed as a single disease, CRC more accurately represents a constellation of heterogeneous subtypes that result from different combinations of genetic events and epigenetic alterations. A growing body of evidence supports the ability to aggregate CRC subtypes based on combinations of microsatellite instability (MSI), CpG island methylator phenotype (CIMP), somatic *BRAF* mutation, and/or somatic *KRAS* mutation status.^{2–11} For example, compared with MSS/MSI-low tumors, MSI-high tumors are more likely to be located in the proximal colon, lower stage, higher grade, and associated with increased tumor-infiltrating lymphocytes.^{12,13} CIMP-positive (or CIMP-high) tumors have been associated with older age, proximal colonic location, poor differentiation and MSI-high status.^{3,9,14,15} Outside of familial syndromes, somatic *BRAF* mutation (exon 15, V600E) appears to be strongly correlated with CIMP-positive or CIMP-high tumors.^{3,16} Somatic *KRAS* mutations (particularly in codons 12 and 13) are reportedly more common in CIMP-positive tumors with a lesser degree of hypermethylation (CIMP low), and have also been associated with the MSS/MSI-low and *BRAF*-mutation –negative CRC subtypes.^{3,17}

To further clarify the complex relationships among MSI, CIMP, *BRAF*, and *KRAS* status in colorectal carcinogenesis, several integrated molecular models have been described previously.^{11,18–20} In a recent special issue of *Gastroenterology* dedicated to CRC updates and future directions, Leggett and Whitehall proposed the following predominant pathways for sporadic CRC development, building from existing integrated models and further incorporating the timing of critical molecular alterations¹¹: the traditional pathway, characterized by early *APC* mutation and chromosomal instability, resulting in MSI-low or MSS, CIMP-negative, *BRAF*-mutation–negative, and *KRAS* mutation–negative tumors; the alternate pathway, in which either *KRAS* or *APC* mutation precedes development of MSI-low or MSS, CIMP-low tumors; and the serrated pathway, in which *BRAF* mutation can lead to CRCs with MSI-high, CIMP-high, or MSI-low or MSS, CIMP-high phenotype.

At present, anatomic extent of disease (as represented by TNM stage) is the most commonly employed measure for estimating CRC prognosis.^{21,22} Yet, the TNM system²³ does not adequately account for within-stage, sub-histologic heterogeneity, prompting recommendations for additional assessment of molecular markers as adjuncts to, or modifiers of, TNM staging.^{21,22,24–26} To date, the traditional, alternate, and serrated

pathways have not been characterized with respect to their clinicopathologic associations or prognostic potential in prospective, population-based studies. Data and tissue resources from the Iowa Women's Health Study (IWHS) of older women were used to generate novel data in this regard.

Materials and Methods

Approvals for the current study were obtained from the Institutional Review Boards for Human Research at Mayo Clinic Rochester, the University of Minnesota and the University of Iowa.

Cohort Recruitment and Case Ascertainment

Details regarding the methods used for recruitment and enrollment of IWHS participants have been reported elsewhere.²⁷ In brief, in January 1986, a 16-page baseline questionnaire was sent to 99,826 randomly selected women, ages 55–69 years, who resided in Iowa and held a valid driver's license. Of these, 41,836 women (42%) returned the baseline questionnaire, constituting the full IWHS subject cohort. Incident CRC cases were identified through the State Health Registry of Iowa, which participates in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program.²⁸ Annual matching between a computer-generated list of all cohort members and the records of Iowans with incident cancer in the SEER program registry was performed based on combinations of first, last, and maiden names; ZIP code; birth date; and social security number. Demographic characteristics and CRC incidence rates for baseline survey responders and nonresponders have been shown to be similar, as reported previously.²⁹ Data on tumor location, grade, SEER stage, chemotherapy exposure, and radiation therapy exposure were obtained from the Iowa registry. CRCs located in the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure (ICD-O codes 18.0, 18.2–18.5) were categorized as proximal colon and cancers located in the descending colon, sigmoid colon, rectosigmoid junction and rectum (ICD-O codes 18.6, 18.7, 19.9, 20.9) were categorized as distal colon or rectum.

Mortality Data

Vital status and state of residence for IWHS participants were determined by mailed questionnaires in 1987, 1989, 1992, 1997, and 2004, as well as through linkage to Iowa death certificate records. Nonrespondents to follow-up surveys were compared against the National Death Index to identify decedents. Previous studies have estimated that 99% of all cancer-related deaths among IWHS cohort members are captured through this approach.³⁰

Tissue Collection and Processing

Archived, paraffin-embedded tissue specimens were requested for incident CRC cases diagnosed from January 1, 1986 through December 31, 2002. For each participant, the pathology laboratory of record was contacted through an introductory request letter, with a second request letter and additional telephone request as needed. Pathology reports, diagnostic slides, and tissue blocks were mailed directly to Iowa Cancer Registry staff for initial accessioning, followed by shipment to the study laboratory coordinator (LST) at Mayo Clinic Rochester. Confirmation of CRC diagnosis and tissue block selection were performed for each case by an experienced gastrointestinal pathologist (TCS). Tissue specimens were retrieved from 732 of 1255 (58%) incident CRC cases. Of note, similar or lower incident CRC case numbers and/or retrieval rates have been recently reported in molecular epidemiology studies embedded within other large cohorts, such as the Nurses' Health Study (n = 528 [58%])³¹ and the Health Professionals Follow-Up Study (n = 438 [51%]).³² To assess the possibility of selection bias, associations among subject

demographics, exposure patterns, and tumor characteristics (size and stage) were compared between patients whose tissue specimens could be retrieved and those whose specimens could not be retrieved. No statistically significant differences were observed for any comparisons, as reported previously.³³ Tissue sections were serially cut in 5- or 10- μ m thick increments. H&E staining was used to identify areas of tumor (ie, >50% dysplastic cells in field of view) and normal tissue. In total, pathology materials were retrieved for 732 incident CRC cases. Tissue samples were scraped from unstained slides and placed into separate tubes. DNA extraction was performed using the QIAamp tissue kit (Qiagen, Valencia, CA) in accordance with manufacturer's instructions. High-quality, usable DNA samples were available from 563 of 732 (77%) cases.

Molecular Markers

MSI status was determined from paired tumor and normal DNA samples using 10 established microsatellite markers: 4 mononucleotide repeats (BAT25, BAT26, BAT40, and BAT34C4), 5 dinucleotide repeats (ACTC, D5S346, D18S55, D17S250, and D10197), and 1 complex marker (MYCL). Polymerase chain reaction (PCR) for the various microsatellite markers was carried out under standard conditions (95°C for 12 min followed by 38 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension for 10 min at 72°C) with a master mix that included 10 $\times$ buffer type II, *Taq* gold, and all 4 deoxyribonucleotide triphosphates. Primers were custom ordered with various fluorescent dyes from Applied Biosystems (Foster City, CA). PCR products were analyzed on an ABI 3100 (Applied Biosystems). MSI status was categorized as MSI high if at least 3 of 10 markers demonstrated instability, MSI low if 1 or 2 of 10 markers demonstrated instability, microsatellite stable (MSS) if 0 of 10 markers demonstrated instability, and MSI missing if assay results were noninformative/unavailable.

CIMP status was evaluated by treating tumor DNA with sodium bisulfite (Zymo Research, Orange, CA) and subsequently analyzed using an automated real-time, PCR-based MethyLight system, which quantitatively measures genome-specific DNA methylation levels in comparison with a methylated reference sample (M.SssI-treated DNA) to calculate the percentage of methylated reference value for each sample and gene region. PCR primers and reaction components were obtained from Applied Biosystems and from Biosearch Technologies (Novato, CA) to amplify methylated CpG sites in the promoter regions of an established 5-gene marker panel (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOC3*).³ CRC cases were categorized as CIMP high if DNA hypermethylation (PMR > 10) was detected in at least 3 of 5 genes, CIMP low if DNA hypermethylation was detected in 1 or 2 of 5 genes, CIMP negative if DNA hypermethylation was detected in 0 of 5 genes, and CIMP missing if assay results were noninformative/unavailable. Classification of CRC tumors into CIMP-high, CIMP-low, and non-CIMP subgroups using MethyLight reactions was first presented by Ogino and colleagues using a slightly modified 5-gene marker panel.³⁴ Subsequently, these CIMP-based subgroups of CRC were also identified using the genome-scale Illumina HumanMethylation27 BeadArray technology in 2 recent reports.^{35,36}

Somatic *BRAF* mutation status was analyzed using fluorescent allele-specific PCR to detect the V600E point mutation in exon 15. In brief, a multiplex PCR containing forward primers for the wild-type sequence and for the V600E alteration, along with a common reverse primer, was carried out on tumor DNA for each incident CRC case. Thermocycler conditions were 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 56°C for 40 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Primers were custom ordered with fluorescent dyes from Applied Biosystems and the PCR product was analyzed on an ABI 3100 (Applied Biosystems). Somatic *BRAF* mutation status was categorized as positive, negative, or missing if the V600E point mutation was detected, not detected, or assay results were non-informative/unavailable, respectively.

Somatic *KRAS* mutation status was analyzed using PCR sequencing to detect well-described point mutations in codons 12 and 13.^{37,38} The clinical relevance and analytic methods for other *KRAS* mutations, such as those occurring in codon 61, are less well established³⁹ and were, therefore, not included in the current study. In brief, a multiplex PCR containing forward primers, along with a common reverse primer, was carried out on tumor DNA for each incident CRC case. Thermocycler conditions were 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 56°C for 40 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Somatic *KRAS* mutation status was categorized as positive, negative, or missing if the analyzed point mutations were detected, not detected, or assay results were non-informative/unavailable, respectively.

Integrated Pathways

Molecular marker data were combined to differentiate CRC cases based on the integrated carcinogenic pathway model proposed by Leggett and Whitehall (although not explicitly stated in the published model, serrated pathway tumors were assumed to be *KRAS* mutation negative and alternate pathway tumors were assumed to be *BRAF* mutation negative).¹¹ CRC cases were then classified as traditional pathway tumors (MSS, CIMP negative, *BRAF* mutation negative, and *KRAS* mutation negative), serrated pathway tumors (any MSI status, CIMP high, *BRAF* mutation positive, and *KRAS* mutation negative), or alternate pathway tumors (MSS, CIMP low, *BRAF* mutation negative, and *KRAS* mutation positive). For cases with noninformative/unavailable data for any of the analyzed markers, pathway assignments were accepted if data were available for at least 2 markers and the available data were consistent with the traditional, serrated, or alternate pathway combinations. For example, a CRC case with MSI high and *BRAF*-mutation-positive status, with missing CIMP and *KRAS* data, was assigned to the serrated pathway. Among CRC cases for which a pathway could not be assigned, 2 dominant clusters were identified, hereafter referred to as cluster A (MSS or MSI low, CIMP negative, *BRAF* mutation negative, and *KRAS* mutation positive) and cluster B (MSS or MSI low, CIMP low, *BRAF* mutation negative, and *KRAS* mutation negative).

Statistical Analysis

Data were descriptively summarized using frequencies and percentages for categorical variables and means and SDs for continuous variables. We compared distributions of demographic and clinical variables in CRC cases across integrated carcinogenic pathways using χ^2 tests for categorical variables and analyses of variance for continuous variables.

Post-CRC follow-up was calculated as the time from initial CRC diagnosis to death or end of the defined study period (December 31, 2010). We compared associations of individual biomarkers and integrated carcinogenic pathways with risk of death using Kaplan-Meier curves. Two sets of survival analyses were carried out: one based on all-cause mortality and one based on CRC-specific mortality. For the latter analyses, subjects dying of causes unrelated to CRC were censored at the date of death.

Cox proportional hazards regression analyses were used to estimate biomarker- and pathway-specific survival hazard ratios and 95% confidence intervals (CIs). Two sets of Cox models were fit: one adjusting for age at CRC diagnosis alone and one adjusting additionally for anatomic subsite (proximal colon, distal colon, or rectum); tumor grade (1, 2, or 3/4); SEER stage (local, regional, or distant); chemotherapy exposure (no or yes); and radiation therapy exposure (no or yes). All statistical tests were 2-sided, and all analyses were carried out with the SAS proprietary software (release 9.2 [TS2M3] for Linux; R version 2.14.0; Vienna, Austria).

Results

For the 563 incident CRC cases with available molecular marker data, the observed distributions by independent marker status were: MSI high = 148 (26%), MSI low = 118 (21%), and MSS = 282 (50%) cases (unable to determine MSI status for 15 [3%] cases); CIMP high = 167 (30%), CIMP low = 91 (16%), and CIMP negative = 277 (49%) cases (unable to determine CIMP status for 28 [5%] cases); *BRAF* mutation positive = 154 (27%) and *BRAF* mutation negative = 391 (69%) cases (unable to determine *BRAF* mutation status for 18 [3%] cases); and *KRAS* mutation positive = 168 (30%) and *KRAS* mutation negative = 347 (62%) cases (unable to determine *KRAS* mutation status for 48 [9%] cases).

In total, we were able to assign 370 CRC cases (66%) to one of the defined integrated pathways, with the following distributions: traditional pathway = 170 cases (46%), alternate pathway = 58 cases (16%), and serrated pathway = 142 cases (38%). The remaining 193 cases (34%) could not be assigned to 1 of the 3 defined integrated pathways. However, there were sufficient nonmissing data to assign 2 new clusters, termed *cluster A* and *cluster B* (as described in the Materials and Methods section), with the following distributions: cluster A = 96 cases (50%) and cluster B = 25 cases (13%). Case numbers for each possible combination of MSI, CIMP, *BRAF* mutation, and *KRAS* mutation status are provided as Supplementary Material, along with the corresponding integrated pathway assignments (Supplementary Table 1).

Mean age at initial CRC diagnosis was 73.9 years overall and increased slightly across the traditional, alternate, and serrated pathways ($P = .03$; Table 1). Integrated pathway assignments were also statistically significantly associated with year of CRC diagnosis (alternate and serrated pathways were less common in the earlier years of the study; $P = .02$); anatomic subsite (serrated pathway was more commonly seen in the proximal colon; $P < .001$); and tumor grade (serrated pathway was more commonly seen in high grade tumors; $P < .001$).

Mean (SD) survival time for all molecularly analyzed CRC cases was 8.04 years (6.11 years). All-cause mortality was not significantly associated with any of the molecularly defined CRC subtypes (Table 2). Kaplan-Meier curves were generated by independent marker status (Figure 1A–D for all-cause mortality; Figure 2A–D for CRC mortality) and integrated pathway assignments (Figure 3A–D). Based on Cox regression analyses, CRC mortality was lower for MSI-high vs MSS cases in both age-adjusted (relative risk [RR] = 0.39; 95% CI: 0.25–0.62) and multivariate-adjusted (RR = 0.54; 95% CI: 0.30–0.98) risk models. Conversely, *KRAS*-mutation –positive vs *KRAS*-mutation –negative tumors exhibited increased CRC mortality in the age-adjusted models (RR = 1.41; 95% CI: 1.03–1.94), although the risk estimate was no longer statistically significant after accounting for additional covariates (RR = 1.40; 95% CI: 0.95–2.06). Neither CIMP status nor *BRAF* mutation status were related to CRC mortality overall, in either the age- or multivariate-adjusted risk models. However, when stratified by MSI status, *BRAF*-mutation –positive tumors were associated with significantly higher all-cause mortality and CRC mortality in the subset of MSI-low cases (RR = 4.05; 95% CI: 1.9–8.61; $P < .001$ and RR = 6.18; 95% CI: 2.31–16.56; $P < .001$ for comparisons to *BRAF*-mutation –negative tumors, respectively), although no statistically significant associations were observed with MSI-high tumors (RR = 0.85; 95% CI: 0.46–1.56; $P = .59$ and RR = 1.29; 95% CI: 0.3–5.63; $P = 7.3$, respectively) or MSS tumors (RR = 1.50; 95% CI: 0.77–2.93; $P = .23$ and RR = 2.03; 95% CI: 0.90–4.58; $P = .09$, respectively).

Analyses based on integrated pathway assignments demonstrated higher CRC mortality risks for alternate and serrated pathway cases compared with traditional pathway cases

(Table 2), but neither of these pathway-defined, multivariate-adjusted risk associations were statistically significant. Interestingly, for the pathway-unassigned cases, cluster A tumors were associated with significantly increased CRC mortality risk (RR = 1.76; 95% CI: 1.07–2.89), although the risk associated with cluster B tumors was elevated to a lesser degree, and was not statistically significant (RR = 1.46; 95% CI: 0.64–3.35).

To account for the possibility of unidentified Lynch syndrome subjects in our CRC case set, 32 tumors with molecular marker combinations of MSI-high and *BRAF*-mutation–negative status (with any CIMP and any *KRAS* mutation status) were excluded in secondary analyses. The resulting survival curves and risk estimates were not statistically significantly different from the primary analyses presented (data not shown).

Discussion

In this prospective cohort study of older women, we found that age at diagnosis, year of diagnosis, anatomic subsite, and histologic grade were statistically significantly associated with some, but not all, molecularly defined CRC subtypes characterized by independent and/or integrated analyses of MSI, CIMP, *BRAF* mutation, and *KRAS* mutation status. MSI phenotype, and, to a lesser degree, *KRAS* mutation status, also appeared to predict CRC mortality, although CIMP, *BRAF* mutation, and traditional, serrated, or alternate pathway designation were not clearly associated with post-CRC survival. These data add to the relative paucity of population-based CRC molecular marker studies and, to our knowledge, represent the first report of clinicopathologic factors and survival outcomes associated with the integrated pathway model recently proposed by Leggett and Whitehall.¹¹

Single molecular markers, including MSI, CIMP, *BRAF*, *KRAS*, *TP53*, and deletion of chromosome 18q, have all been investigated to varying extents as CRC prognostic indicators. MSI status has been evaluated as a potential prognostic indicator in >30 studies to date, with pooled analyses demonstrating a 35%–40% survival benefit for MSI-high vs MSI-low/MSS tumors.^{40,41} Among IWHS subjects, MSI-high tumors were observed in 26% of cases (consistent with the established associations between MSI and older age and/or female sex^{33,42–44}) and were associated with a 46% lower CRC mortality risk than MSS tumors, in keeping with the published pooled analyses. CIMP status has also been investigated as a CRC prognostic marker in a number of earlier studies,^{5,8,14,45–49} with mixed results. In our cohort of older women, the prevalence of CIMP-high and CIMP-low tumors was relatively high (because CIMP status is also known to be positively associated with older age at diagnosis and female sex⁵⁰), but no apparent advantage was observed with respect to CRC-specific mortality for CIMP-high, CIMP-low, or CIMP-negative cases. Discrepant CIMP-associated mortality risks across studies might be attributable to multiple factors, including differences in subject characteristics (eg, age, sex, and race/ethnicity), environmental exposures, analytic methods, marker panels, and type/extent of chemotherapy exposure, among others.

Several additional studies have examined the prognostic potential of *BRAF* status, with general consensus that somatic V600E mutation is associated with adverse clinical outcomes.^{7,8,14,51–57} However, whether or not *BRAF* mutation confers a survival disadvantage independent of other molecular signatures, particularly MSI status, remains unresolved. In the IWHS cohort, isolated *BRAF* evaluation does not seem to provide appreciable benefits for projecting CRC mortality (although *BRAF*-mutation–positive tumors were associated with higher risk among the subset of MSI-low cases, based on a relatively small sample size). Earlier studies of *KRAS* as a prognostic biomarker, conducted before Food and Drug Administration approval of anti-epidermal growth factor receptor monoclonal antibodies for CRC chemotherapy, also provided conflicting results,⁵⁸

consistent with the possible, but not statistically significant, association between *KRAS*-mutation –positive tumors and CRC mortality observed in our temporally congruent study.

The molecular complexity of colorectal tumorigenesis supports the potential utility of assessing multiple markers in combination to predict CRC outcomes. When the IWHS cases were grouped and analyzed by defined integrated carcinogenic pathway assignments, striking associations were noted with anatomic subsite (92% of the serrated tumors were located in the proximal colon) and tumor grade (52% of serrated tumors were grade 3 or 4). Yet, despite these demonstrated differences, no appreciable association was detected between integrated pathway assignment and CRC mortality, for reasons that remain to be clarified.

Potential limitations of our study should be acknowledged. The inability to categorize all CRC cases into pathway-specific subsets likely relates to biologic mechanisms that were not fully described by the assay panels or integrated model used. Interestingly, cluster A tumors (characterized by MSS or MSI-low, CIMP-negative, *BRAF*-mutation –negative, and *KRAS*-mutation –positive status), which represented 50% of the pathway unassigned cases, were associated with the highest CRC mortality risk (RR = 1.76; 95% CI: 1.07–2.89) in our study, suggesting that further evaluation of the molecular alterations underlying this incompletely characterized CRC subset (as well as the cluster B cases) can be informative. Also, although linkage to the Iowa Cancer Registry afforded comprehensive CRC case ascertainment and ready access to well-annotated tissue specimens, we were not able to retrieve adequate tissue specimens from all IWHS subjects with incident CRC for the planned molecular analyses. As noted (see Materials and Methods), no major biases were identified by the tissue procurement or processing methods. Lastly, the reported findings were obtained from older, predominately Caucasian women and neither the CRC subtype distributions nor the clinicopathologic associations can be directly applied to other demographically defined population subgroups without additional investigation.

In conclusion, novel data from our population-based cohort study demonstrate that molecularly defined CRC subtypes (based on independent marker and/or integrated pathway analyses) are associated with distinct clinicopathologic characteristics, at least among older Caucasian women. However, additional evaluation is needed to identify the genetic events and epigenetic alterations that most accurately predict CRC survival outcomes in other population subgroups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

CI	confidence interval
CIMP	CpG island methylator phenotype
CRC	colorectal cancer
IWHS	Iowa Women's Health Study
MSI	microsatellite instability

PCR	polymerase chain reaction
RR	relative risk
SEER	Surveillance, Epidemiology, and End Results

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Figure 1. Kaplan–Meier curves. All-cause mortality by independent marker status. (A) MSI; (B) CIMP; (C) *BRAF* mutation; and (D) *KRAS* mutation.

Figure 2. Kaplan–Meier curves. CRC mortality by independent marker status. (A) MSI; (B) CIMP; (C) *BRAF* mutation; and (D) *KRAS* mutation.

Figure 3. Kaplan–Meier curves. All-cause and CRC mortality by integrated pathway assignments. (A) All-cause mortality by traditional, alternate, serrated, or other (unassigned) pathways. (B) CRC mortality by traditional, alternate, serrated, or other (unassigned) pathways. (C) All-cause mortality in pathway unassigned cases (cluster A, cluster B, or other). (D) CRC mortality in pathway unassigned cases (cluster A, cluster B, or other).

Table 1

Clinicopathologic Characteristics for Molecularly Analyzed CRC Cases in the Iowa Women's Health Study (1986–2002), by Integrated Pathway Assignment

	All cases (n = 563)	Traditional pathway (n = 170)	Alternate pathway (n = 58)	Serrated pathway (n = 142)	Pathway unassigned (n = 193)	P Value ^a
Characteristic						
Age at diagnosis, y, mean (SD)	73.9 (5.92)	73.2 (5.96)	74.6 (5.68)	75.1 (5.74)	73.4 (5.96)	.03
Year of diagnosis, n (%)						.02
1986–1989	51 (9.1)	16 (9.4)	4 (6.9)	7 (4.9)	24 (12.4)	
1990–1999	352 (62.5)	120 (70.6)	36 (62.1)	84 (59.2)	112 (58)	
2000–2002	160 (28.4)	34 (20.0)	18 (31.0)	51 (35.9)	57 (29.5)	
Anatomic subsite, n (%)						<.0001
Proximal colon	317 (56.9)	46 (27.2)	31 (53.4)	130 (92.2)	110 (58.2)	
Distal colon	145 (26.0)	74 (43.8)	14 (24.1)	8 (5.7)	49 (25.9)	
Rectum	95 (17.1)	49 (29.0)	13 (22.4)	3 (2.1)	30 (15.9)	
Unavailable/unknown, n	6	1	0	1	4	
Tumor grade, n (%)						<.0001
1	32 (5.9)	9 (5.5)	5 (8.9)	4 (2.9)	14 (7.6)	
2	340 (62.5)	115 (69.7)	41 (73.2)	62 (44.9)	122 (65.9)	
3 or 4	172 (31.6)	41 (24.8)	10 (17.9)	72 (52.2)	49 (26.5)	
Unavailable/unknown, n	19	5	2	4	8	
SEER stage, n (%)						.14
Localized	188 (37.8)	64 (41.3)	24 (43.6)	35 (29.4)	65 (38.5)	
Regional metastases	237 (47.6)	70 (45.2)	22 (40.0)	69 (58.0)	76 (45.0)	
Distant metastases	73 (14.7)	21 (13.5)	9 (16.4)	15 (12.6)	28 (16.6)	
Unavailable/unknown, n	65	15	3	23	24	
Chemotherapy exposure, n (%)						.08
No	431 (76.6)	119 (70.0)	47 (81.0)	113 (79.6)	152 (78.8)	
Yes	132 (23.4)	51 (30.0)	11 (19.0)	29 (20.4)	41 (21.2)	
Unavailable/unknown, n	0	0	0	0	0	
Radiation exposure, n (%)						.11
No	527 (95.8)	155 (93.9)	51 (94.4)	140 (98.6)	181 (95.8)	
Yes	23 (4.2)	10 (6.1)	3 (5.6)	2 (1.4)	8 (4.2)	
Unavailable/unknown, n	13	5	4	0	4	

^aReported P values are based on analysis of variance for continuous variables and ² tests for categorical variables.

Table 2

Associations Between Independent Markers, Integrated Pathways, and Mortality Among CRC Cases in the Iowa Women's Health Study (1986–2002)

	Person-years	All-cause mortality			CRC mortality		
		Deaths	HR (95% CI) ^a	HR (95% CI) ^b	Deaths	HR (95% CI) ^a	HR (95% CI) ^b
Independent markers							
MSS	2,222	187	1.00 (ref)	1.00 (ref)	103	1.00 (ref)	1.00 (ref)
MSI low	906	74	0.98 (0.75–1.29)	1.03 (0.76–1.41)	43	1.02 (0.71–1.45)	0.98 (0.65–1.49)
MSI high	1,243	90	0.84 (0.65–1.08)	1.01 (0.71–1.42)	22	0.39 (0.25–0.62)	0.54 (0.30–0.98)
<i>P</i> value			.19	.92		<.001	.08
CIMP negative	2,335	176	1.00 (ref)	1.00 (ref)	89	1.00 (ref)	1.00 (ref)
CIMP low	694	58	1.05 (0.78–1.42)	0.86 (0.60–1.23)	31	1.10 (0.73–1.66)	1.19 (0.72–1.97)
CIMP high	1,223	107	1.08 (0.84–1.37)	1.12 (0.81–1.55)	45	0.94 (0.66–1.35)	1.06 (0.65–1.72)
<i>P</i> value			.55	.60		.80	.74
BRAF mutation negative	3,257	250	1.00 (ref)	1.00 (ref)	124	1.00 (ref)	1.00 (ref)
BRAF mutation positive	1,062	102	1.16 (0.92–1.47)	1.22 (0.89–1.68)	46	1.10 (0.78–1.55)	1.23 (0.78–1.94)
<i>P</i> value			.20	.21		.59	.38
KRAS mutation negative	2,797	219	1.00 (ref)	1.00 (ref)	97	1.00 (ref)	1.00 (ref)
KRAS mutation positive	1,234	112	1.15 (0.92–1.44)	1.05 (0.80–1.38)	63	1.41 (1.03–1.94)	1.40 (0.95–2.06)
<i>P</i> value			.23	.70		.03	.08
Integrated pathways							
Traditional	1,532	109	1.00 (ref)	1.00 (ref)	51	1.00 (ref)	1.00 (ref)
Alternate	443	39	1.17 (0.81–1.68)	0.85 (0.55–1.32)	19	1.21 (0.71–2.05)	1.26 (0.66–2.42)
Serrated	1,045	92	1.13 (0.85–1.49)	1.23 (0.84–1.78)	37	1.02 (0.67–1.56)	1.56 (0.88–2.77)
Unassigned	1,509	121	1.08 (0.83–1.40)	1.08 (0.80–1.48)	65	1.23 (0.85–1.77)	1.52 (0.97–2.39)
Cluster A ^c	722	62/722	1.14 (0.84–1.56)	1.16 (0.81–1.65)	36/722	1.41 (0.92–2.17)	1.76 (1.07–2.89)
Cluster B ^c	193	14/193	0.97 (0.55–1.69)	0.98 (0.52–1.85)	9/193	1.24 (0.61–2.52)	1.46 (0.64–3.35)
<i>P</i> value			.92	.70		.64	.32

NOTE. Reported *P* values are based on trend across marker levels, except for the integrated pathway analyses (χ^2 test with 5 degrees of freedom).

HR, hazard ratio.

^aAdjusted for age at diagnosis.

^bAdjusted for age at diagnosis, anatomic subsite, tumor grade, SEER stage, chemotherapy exposure, and radiation therapy exposure.

^cClusters A and B defined in the Materials and Methods section.