Genomic Aberrations Occurring in Subsets of Serrated Colorectal Lesions but not Conventional Adenomas

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Abstract

A subset of aggressive colorectal cancers exhibit BRAF mutation, MLH1 methylation, and a CpG island methylator phenotype (CIMP), but precursors are poorly established. In this study, we determined the status of these markers in colorectal polyps and evaluated associated risk factors. The study included 771 polyp cases and 1,027 controls who were ages 24 to 80 years, part of a group health program, received a colonoscopy from 1998 to 2007, and completed a structured questionnaire assessing risk factors. Following standard pathology review, polyps were assayed for BRAF mutation (V600E) and tested for MLH1 and CIMP methylation, the latter including the genes, CACNA1G, IGFl, NEUROGI1, RUNX3, and SOCSI. Polytomous logistic regression was used to estimate ORs and 95% confidence intervals for the association between molecularly defined subsets of polyps and potential risk factors. There were 580 conventional adenomas and 419 serrated lesions successfully assayed. For adenomas, the prevalence of each marker was ≤1%. In contrast, 55% of serrated lesions harbored mutant BRAF, 26% were CIMP-high, and 5% had methylated MLH1. In these lesions, the highest prevalence of markers was in sessile-serrated polyps (SSP) of ≥10 mm that were in the right-side/cecal regions of the colon. Risk factors for CIMP-high–serrated lesions included Caucasian race, current smoking status, and a history of polyps, whereas for serrated lesions with mutant BRAF, the significant risk factors were male sex, current smoking status, obesity, and a history of polyps. Our results suggest that SSPs and other large, right-sided serrated lesions have a unique molecular profile that is similar to CIMP-high, BRAF-mutated colorectal cancers. Cancer Res; 73(9); 2863-72. ©2013 AACR.

Introduction

Colorectal cancer (CRC) can form via a variety of pathways, resulting in tumors with distinct phenotypic and molecular characteristics (1). The adenoma–carcinoma pathway gives rise to approximately 75% of CRCs (2). Carcinomas developing from this pathway tend to exhibit chromosomal instability (CIN) and lack microsatellite instability (MSI; ref. 3). Even though conventional colorectal adenomas are well-established precursor lesions in this pathway, generally few will progress to cancer (4); however, conventional adenomas ≥10 mm in diameter have a much greater risk of developing into malignant tumors than those <10 mm (5).

A second pathway for colorectal carcinogenesis, termed “the serrated pathway,” commonly involves a high frequency of aberrant promoter related CpG island methylation, which has been called the CpG island methylator phenotype (CIMP; ref. 6). Serrated neoplasms frequently carry methylated MLH1, which is one of the mismatch repair genes. The methylation of MLH1 results in a form of genomic instability called MSI. This pathway is also closely linked to the somatic BRAF p.V600E mutation, which results in increased cellular proliferation through abnormal mitogen-activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) signaling (7, 8). Thus, the serrated pathway results in carcinomas characterized as CIMP-high that are often MSI-high and carry mutant BRAF (6).

A class of polyps termed serrated lesions are commonly CIMP-high and carry mutant BRAF (9); therefore, they are hypothesized to be important in the serrated pathway for CIMP-high CRC (6, 9). Serrated lesions include goblet cell hyperplastic polyps, microvesicular hyperplastic polyps, sessile-serrated polyps (SSP; also known as sessile-serrated adenomas), and traditional serrated adenomas (TSA; ref. 10). TSAs tend to occur in the distal colon and rectum and are characterized as having cytologic dysplasia, a serrated appearance, and ectopic crypts (11). Because of the dysplastic phenotype of these polyps, they are viewed as potential precursor lesions (11). In contrast, most hyperplastic polyps and SSPs are not dysplastic and traditionally have been considered to have no malignant potential. However, new evidence suggests that...
SSPs may be precursors for CIMP-high CRC, particularly in the proximal colon (9).

Despite tremendous progress made over the past decade in understanding serrated lesions and the serrated pathway to CRC, much about this pathway remains unclear. Thus, we aimed to characterize colorectal conventional adenomas, hyperplastic polyps, SSPs, and TSAs in terms of BRAF mutation, CIMP, and MLH1 methylation. In addition, we evaluated risk factors for BRAF mutation and CIMP.

Materials and Methods

Study population

Participants, ages 20 to 79 years, were enrollees of Group Health (GH), an integrated healthcare provider in Washington State, who underwent an index colonoscopy for any indication between 1998 and 2007, and were diagnosed on the basis of medical records, those who agreed to participate and those who refused study participation were similar with respect to age, sex, and colorectal polyp status. Study protocols were approved by the Institutional Review Boards of GH and the Fred Hutchinson Cancer Research Center (Seattle, WA). Additional details of the study population have been previously reported (12, 13).

Study questionnaire

Participants completed a structured questionnaire that elicited information on demographics, personal and family medical history, height, weight, nonsteroidal anti-inflammatory drug (NSAID) use, and cigarette smoking. Exposure ascertainment was limited to experiences that occurred ≥1 year before the index colonoscopy. Data collection took place in 2 phases, and similar questionnaires were used in both phases. During phase I, participants were identified from a listing of patients undergoing colonoscopy from September 1998 to March 2003, and participants were generally interviewed before the index colonoscopy. During phase II, which included patients receiving colonoscopy December 2004 and September 2007, study participants were interviewed 3 to 4 months following the index colonoscopy, and right-sided serrated lesions were oversampled. Controls had no colorectal polyps identified during the index colonoscopy and were systematically sampled to reflect the age (within a 5-year range) and calendar year of polyp cases.

Polyp classification

Diagnostic biopsies collected at the index colonoscopy were stored in formalin-fixed, paraffin-embedded (FFPE) blocks; sections were cut onto slides and stained with hematoxylin and eosin. Two study pathologists (M. Upton and L. Zhu) worked in tandem to conduct a standardized pathology review of polyp tissue slides. Disagreements in diagnoses between study pathologists were reconciled through re-review by both pathologists. Conventional adenomas included tubular adenomas, tubulovillous adenomas, and villous adenomas. Serrated lesions included goblet cell hyperplastic polyps, microvillous hyperplastic polyps, TSAs, and SSPs (14). SSPs were distinguished from other serrated lesions if they displayed exaggerated crypt serration, crypt dilatation, crypt branching, horizontal crypt extensions at the base, or other distortion of architectural organization and maturation (15). Anatomic location and polyp size were abstracted from the electronic medical record.

Tissue sample selection and preparation

We selected FFPE polyp tissue blocks for analysis if the polyp or tissue sample was ≥3 mm in size based on clinical diagnosis and the block contained ≥50% lesional tissue. Samples were labeled with anonymized identification numbers, and laboratory technicians were blinded to polyp diagnosis. DNA was extracted from FFPE tissue using the Qiagen FFPE Tissue DNA Extraction Kit (Qiagen) and quantified using Picogreen (Invitrogen), as previously described (12).

Tissue testing for BRAF mutation, MLH1 methylation, and CIMP status

Polyp tissue DNA was treated with sodium bisulfite using the Zymo EZ DNA Methylation Kit (Zymo Research). CIMP status and MLH1 methylation were determined using the MethyLight assay as described in Weisenberger and colleagues (16). The CIMP panel included the following 5 markers: CACNA1G, IGF2, NEUROG1, RUNX3 and SOCS1. The primer and probe sequences have been previously published (16). The output from this assay was percent methylated relative to a DNA reference sample (PMR). Using pre-established and validated criteria, samples having ≥3 markers with PMR > 10 were considered CIMP-high, others were CIMP-low/negative (16). For MLH1, samples with PMR > 10 for this gene were considered to have methylated MLH1. Positive and negative controls were run on each plate, and ALU was used to monitor sample quality. On the basis of pilot testing, we considered samples to be insufficient if the ALU cycle threshold value (Ct) was >23. Such samples were retested with higher input volumes and, if ALU Ct remained >23, the sample was considered to have failed the MethyLight assay. Performance characteristics of the MethyLight assay were previously assessed (17).

Two methods were used to assess BRAF p.V600E mutation status in polyp tissue DNA samples. All samples were tested using TaqMan PCR as described by Benlloch and colleagues (18). Samples that were not called automatically by the SDS allelic discrimination software from ABI were analyzed real-time and clustered by Ct value. Those samples that remained undetermined with real-time data were considered to have failed the TaqMan PCR assay. Those that failed this assay were also tested using a fluorescent allele-specific PCR assay that included the following primers tagged with differing
fluorophores: mutant forward, 6FAM-5'-CAGTGTATTTGTCTAGCTTCAGA-3'; wild-type forward, NED-5'-TGGATTTTGCTCTAGCTACAGT-3'; and a common reverse primer, 5'-CTCAATTCTTACCATCCAAAATG-3', as described by Buchanan and colleagues (19). The fluorescent PCR products were separated on a 3130xl genetic analyzer (Applied Biosystems) and analyzed using GeneMapper software (Applied Biosystems). Positive and negative controls were run on each plate. Samples were considered to have failed this assay if the wild-type peak did not measure above the fluorescence threshold set in GeneMapper 4.0.

Statistical analyses

The prevalence of BRAF mutation, MLH1 methylation, and CIMP was calculated for adenomas and serrated lesions, overall. Among serrated lesions, the prevalence of each molecular marker was determined according to the following characteristics: (i) histologic type (goblet cell hyperplastic polyp, microvesicular hyperplastic polyp, SSP, and TSA); (ii) anatomic site (rectum, rectosigmoid, left colon [including sigmoid], desmophlegic hyperplastic polyp, SSP, and TSA); (iii) polyp size (3–5, 6–9, and ≥10 mm in diameter). The \( \chi^2 \) values were calculated to assess the statistical significance of differences in the distribution of each molecular marker according to histologic type, anatomic site, and polyp size.

Polymorphic logistic regression, clustered by participant to account for individuals with more than one polyp, was used to estimate adjusted ORs and 95% confidence intervals (CI) comparing polyp cases, with and without each molecular marker, with polyp-free controls (20). These same models were used to compare cases with polyps containing different molecular markers to one another (e.g., wild-type BRAF vs. mutant BRAF, and CIMP-low/negative vs. CIMP-high). The Wald \( P \) value for the comparison between case groups was calculated for each risk factor. Adjustment variables and variables of interest were selected \textit{a priori} on the basis of prior studies reporting an association between each factor and risk of colorectal neoplasia. Regression analyses were adjusted for study phase, age, sex, education, body mass index (BMI; kg/m\(^2\)), regular NSAID use (≥2 doses per week for 12 continuous months), family history of CRC (≥1 first-degree relative with CRC), history of prior polyps, and cigarette smoking status. Approximately 3\% (\( n = 49 \)) of observations were missing data on one or more of these exposure variables and were excluded from analyses. Study participants with missing data were not significantly different from other study participants with respect to the variables of interest (data not shown). Variables were categorized as shown in Table 1. All statistical analyses were conducted using STATA (version 11.0, StataCorp LP).

Results

A total of 2,467 clinical biopsies underwent a standard pathology review and were categorized as a conventional adenoma (tubular adenoma, \( n = 1,115 \); tubulovillous/villous adenoma, \( n = 163 \)) or a serrated lesion (goblet cell hyperplastic polyp, \( n = 94 \); microvesicular hyperplastic polyp, \( n = 815 \); TSA, \( n = 206 \); SSP, \( n = 260 \)). Of these, 1,322 polyps were excluded because the polyp case met the exclusion criteria (i.e., prior history of cancer, ulcerative colitis, etc.) or because of small polyp size (<3 mm), low percent lesional tissue in the biopsy (<50% lesional), or low DNA yield (<20 ng).

Prevalence of BRAF mutation, MLH1 methylation, and CIMP

A total of 1,145 polyps from 909 cases were assayed for BRAF mutation, CIMP, and MLH1 methylation status. Of these, 146 samples (13%) from 138 cases failed one or both assays. Of the remaining 999 polyps, 580 were adenomas and 419 were serrated lesions. Compared with polyp-free controls, cases with conventional adenomas and/or serrated lesions were more likely to be men, have BMI ≥ 30 kg/m\(^2\), be current smokers, and have a history of prior colorectal polyps (Table 1).

In adenomas, BRAF mutation was present in 1%, and CIMP and MLH1 methylation were present in <1%. In contrast, 55% of serrated lesions carried mutant BRAF, 26% were CIMP-high, and 5% had methylated MLH1 (Table 2). Analyses of polyps by histologic subtype, anatomic site, and size were restricted to serrated lesions.

Histologic type

There was significant variation in the prevalence of each molecular marker according to histologic type (\( P < 0.01 \) for each; Fig. 1). The prevalence of serrated lesions with mutant BRAF was lowest in goblet cell hyperplastic polyps (12%), followed by TSAs (45%), microvesicular hyperplastic polyps (53%), and SSPs (68%). SSPs also had the highest prevalence of CIMP (49%) and MLH1 methylation (11%). In contrast, CIMP and MLH1 methylation were absent in goblet cell hyperplastic polyps. There was also no methylated MLH1 in TSAs but 27% were CIMP-high. Microvesicular hyperplastic polyps had low prevalence of CIMP (15%) and methylated MLH1 (3%).

Anatomic site

Information on anatomic site was available from medical records on 418 successfully assayed serrated lesions. There was significant variation in the prevalence of each molecular marker according to anatomic site (\( P < 0.01 \) for each; Fig. 2). BRAF mutations were observed in serrated lesions throughout the rectum and colon, with the right colon and cecum having the highest prevalence of serrated lesions with mutant BRAF (65% and 61%, respectively). The prevalence of CIMP and MLH1 methylation increased in serrated lesions from the rectum (1% CIMP-high and 0% MLH1-methylated) to the cecum (57% CIMP-high and 11% MLH1-methylated).

Lesion size

Size was available for 276 successfully assayed serrated lesions. There was significant variation in the prevalence of CIMP and mutant BRAF according to polyp size (\( P < 0.01 \) for each); however, differences in the distribution of MLH1 methylation according to polyp size were not significant (\( P = 0.07 \); Fig. 3). The prevalence of each molecular marker was highest in serrated lesions ≥10 mm in diameter; 74% of these had mutant BRAF, 46% were CIMP-high, and 11% were MLH1-methylated. In contrast, for serrated lesions 3 to 5 mm in
diameter, 50% had mutant \textit{BRAF}, 20% were CIMP-high, and 4% were \textit{MLH1}-methylated (Fig. 3).

\textbf{Risk factors for CIMP-high and mutant \textit{BRAF} in serrated lesions}

A total of 1,386 study participants, comprising 1,027 polyp-free controls and 359 serrated lesion cases, were included in analyses of risk factors for CIMP-high and mutant \textit{BRAF} in serrated lesions (Table 3). Among the 359 cases, there were 419 serrated lesions; 311 were CIMP-low/negative, 108 were CIMP-high, 187 had wild-type \textit{BRAF}, and 232 had mutant \textit{BRAF}.

Comparing cases with CIMP-high serrated lesions with polyp-free controls, the following risk factors were associated with a statistically significant increase in the odds of CIMP-high serrated lesions: Caucasian race (OR, 2.27; 95% CI, 1.08–4.76), current smoking status (OR, 2.71; 95% CI, 1.19–6.18), and a history of prior colorectal polyps (OR, 2.35; 95% CI, 1.46–3.79).

For CIMP-low/negative serrated lesions, male sex (OR, 1.45; 95% CI, 1.06–1.97), BMI \( \geq 30 \) kg/m\(^2\) (OR, 1.78; 95% CI, 1.21–2.62), current smoking status (OR, 4.41; 95% CI, 2.55–7.60), and a history of prior polyps (OR, 1.44; 95% CI, 1.02–2.04) were all statistically significant risk factors. None of the other risk factors evaluated were statistically significantly associated with CIMP-high or CIMP-low/negative serrated lesions. Only the association with age was statistically significantly different between CIMP-high and CIMP-low/negative serrated lesions (\( P_{\text{heterogeneity}} = 0.03 \)), with CIMP-high cases tending to be older than CIMP-low/negative cases (Table 3).

Compared with polyp-free controls, serrated lesions with mutant \textit{BRAF} were statistically significantly associated with male sex (OR, 1.47; 95% CI, 1.04–2.09), BMI \( \geq 30 \) kg/m\(^2\) (OR, 1.57; 95% CI, 1.03–2.40), current smoking status (OR, 2.75; 95% CI, 1.48–5.11), and history of prior polyps (OR, 1.60; 95% CI, 1.11–2.30; Table 3). The following factors were associated with a statistically significant increase in the odds of serrated lesions with wild-type \textit{BRAF}: BMI \( \geq 30 \) kg/m\(^2\) (OR, 1.93; 95% CI, 1.21–3.09), current smoking status (OR, 5.83; 95% CI, 3.18–10.71), and history of prior polyps (OR, 1.76; 95% CI, 1.17–2.63). No other factors were associated with mutant \textit{BRAF} or wild-type \textit{BRAF} in the serrated lesions, and only the association with

\begin{table}[ht]
\centering
\caption{Characteristics of patients with conventional adenomas, serrated lesions, and controls: Group Health Enrollees 1998–2007}
\begin{tabular}{lcccc}
\hline
 & \multicolumn{2}{c}{Controls} & \multicolumn{2}{c}{Conventional adenomas} & \multicolumn{2}{c}{Serrated lesions} \\
 & \((N = 1027)\) & \((N = 469)\) & \((N = 359)\) \\
\hline
\textbf{Age, y} & & & & & & \\
24–49 & 106 (11) & 19 (4) & 21 (6) \\
50–60 & 455 (44) & 197 (42) & 164 (46) \\
61–70 & 331 (32) & 183 (39) & 141 (39) \\
71–80 & 135 (13) & 70 (15) & 33 (9) \\
\hline
\textbf{Race/ethnicity} & & & & & & \\
Caucasian & 880 (86) & 381 (81) & 318 (89) \\
African American & 37 (4) & 17 (4) & 4 (1) \\
Asian American & 43 (4) & 33 (7) & 11 (3) \\
Other & 67 (6) & 38 (8) & 26 (7) \\
\hline
\textbf{CRC family history (Yes)} & & & & & & \\
High school or less & 155 (23) & 78 (25) & 51 (21) \\
Some college & 253 (38) & 118 (38) & 97 (39) \\
College graduate & 258 (39) & 117 (37) & 100 (40) \\
\hline
\textbf{BMI, kg/m\(^2\)} & & & & & & \\
<25 & 416 (41) & 134 (29) & 126 (35) \\
25–29.9 & 394 (38) & 191 (41) & 132 (37) \\
\geq 30 & 213 (21) & 141 (30) & 100 (28) \\
\hline
\textbf{Smoking status} & & & & & & \\
Never & 573 (56) & 239 (51) & 160 (44) \\
Former & 395 (39) & 191 (41) & 161 (45) \\
Current & 56 (5) & 39 (8) & 38 (11) \\
\hline
\textbf{NSAIDs use (yes)} & & & & & & \\
553 (54) & 236 (51) & 181 (51) \\
\hline
\textbf{Prior polyps (yes)} & 194 (19) & 133 (29) & 104 (29) \\
\hline
\end{tabular}
\footnote{Includes 412 participants with only conventional adenomas and 57 participants with \( \geq 1 \) adenoma and \( \geq 1 \) serrated lesion.}
\footnote{Includes 302 participants with only serrated lesions and 57 participants with \( \geq 1 \) serrated lesion and \( \geq 1 \) conventional adenoma.}
\end{table}
current smoking status was statistically significantly different between serrated lesions with mutant BRAF and wild-type BRAF ($P_{\text{het}} = 0.03$); current smoking status had a stronger association with serrated lesions with wild-type BRAF than with mutant BRAF (Table 3).

**Discussion**

In this large study population, we found that CIMP-high, BRAF mutation, and MLH1 methylation were rarely observed in conventional adenomas (1% or less) but were much more commonly detected in serrated lesions. Thus, our data suggest that serrated lesions may be important precursors for CIMP-high, mutant BRAF carcinomas. In particular, the molecular profiles of SSPs and large, right-sided serrated lesions suggest that these subsets of serrated lesions may be closely linked to CIMP-high, mutant BRAF CRC. Furthermore, although prior CRC studies have used a molecular pathologic epidemiology approach to evaluate risk factors for CIMP and mutant BRAF (21), this is the first study to do so in colorectal polyps. Some of the significant risk factors we observed for CIMP-high and mutant BRAF serrated lesions were previously reported as risk factors for CIMP-high or mutant BRAF CRCs including body size, race/ethnicity, and cigarette smoking (22–25). However, most serrated lesions will not progress to cancer, and in the absence of large longitudinal studies, there is currently a lot of uncertainty about the risk of cancer associated with subsets of serrated lesions. Below we highlight several important findings and discuss our results in relation to prior studies.

**Prevalence of molecular markers by polyp histology**

Before the present study, sample size for studies evaluating CIMP in conventional adenomas, ranged from only 19 to 57 conventional adenomas, with reported prevalence of CIMP that ranged from 18% to 32% (26–29). Our results indicating that only 1% of 580 conventional adenomas were CIMP-high seem at odds with these prior studies. However, the difference between our CIMP-high prevalence estimate and estimates from previous studies may be due to differences in CIMP panels (30). Several different CRC CIMP panels have been used in prior studies, and these distinct panels can yield different results (30). The CIMP panel used in the present study correlates well with mutant BRAF CRC and is therefore hypothesized to identify carcinomas that develop via the serrated pathway (16, 31). Although no other studies of conventional adenomas have used this CIMP panel, 2 prior studies of serrated lesions did use this CIMP-panel, and similar to our results, these studies reported CIMP prevalence of 29% ($n = 52$) and 25% ($n = 17$; refs. 32, 33). Thus, our results suggest that the Weisenberger CIMP panel is specific to serrated lesions; future studies replicating these results in a separate study population would enhance the interpretation of this finding.

In prior studies of both conventional adenomas and serrated lesions, prevalence of BRAF mutation in conventional adenomas ranged from 0% to 2%, and in serrated lesions, it ranged from 50% to 80% (28, 29, 34, 35). Our estimates that 1% of conventional adenomas and 55% of serrated lesions carry mutant BRAF fall within these ranges.

For MLH1 methylation status, prior studies have used different assays and cutoff values to test colorectal polyps; therefore, it is difficult to compare results across studies (28, 29, 32, 33, 36–38). A study by Vaughn and colleagues used the same assay and PMR cutoff as our study and reported that 1 of 52 serrated lesions (2%) were positive for MLH1 methylation (33). This prevalence is consistent with our estimate that 5% of serrated lesions were positive for MLH1 methylation. Also consistent with our study results, some studies reported that the prevalence of MLH1 methylation was higher in serrated lesions than in conventional adenomas (28, 29). However, the other studies reported similar prevalence estimates for MLH1 methylation between serrated lesions and adenomas (36, 37). Additional research is needed to standardize MLH1 methylation

**Table 2. Prevalence of adenomas and serrated lesions with mutant BRAF, CIMP-high, and methylated MLH1: Group Health Enrollees 1998–2007**

<table>
<thead>
<tr>
<th></th>
<th>Conventional adenosas(^a)</th>
<th>Serrated lesions(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N =$ 580</td>
<td>$N =$ 419</td>
</tr>
<tr>
<td>Mutant BRAF</td>
<td>6 (1)</td>
<td>232 (55)</td>
</tr>
<tr>
<td>CIMP-high</td>
<td>4 (1)</td>
<td>108 (26)</td>
</tr>
<tr>
<td>Methylated MLH1</td>
<td>1 (&lt;1)</td>
<td>23 (5)</td>
</tr>
<tr>
<td>Mutant BRAF and CIMP-high</td>
<td>1 (&lt;1)</td>
<td>79 (19)</td>
</tr>
<tr>
<td>Mutant BRAF and methylated MLH1</td>
<td>0 (0)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>CIMP-high and methylated MLH1</td>
<td>0 (0)</td>
<td>19 (4)</td>
</tr>
<tr>
<td>Mutant BRAF and CIMP-high and</td>
<td>0 (0)</td>
<td>14 (3)</td>
</tr>
<tr>
<td>methylated MLH1</td>
<td>None of the above</td>
<td>570 (98)</td>
</tr>
</tbody>
</table>

\(^a\)580 conventional adenomas among 469 participants.

\(^b\)419 serrated lesions among 359 participants.

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![Figure 1. Prevalence of mutant BRAF, CIMP-high, and methylated MLH1 serrated lesions by histologic type: Group Health Enrollees 1998–2008.](image-url)
testing and to determine the clinical significance of MLH1 methylation in colorectal polyps.

Among serrated lesions, all 3 markers were most prevalent in SSPs. Prior studies have also shown high prevalence of BRAF mutation and CIMP in SSPs (7, 28, 34, 39), supporting the thesis that SSPs may be salient precursor lesions for CIMP-high, mutant BRAF CRC (40). Additional evidence supporting SSPs as important precursors includes a histologic study of 8 SSP polypectomies containing focal invasive adenocarcinoma or high-grade dysplasia (41) and a longitudinal study of 40 patients with SSPs at baseline, which found that 12.5% developed CRC within 5 years (42). However, large longitudinal studies are needed to characterize the natural history of, and cancer risk associated with, SSPs.

**Prevalence of molecular markers by anatomic site**

In serrated lesions, we observed different distributions for the methylation-related markers (CIMP and MLH1 methylation) and BRAF mutation according to anatomic site. CIMP and MLH1 methylation occurred rarely outside the right colon and cecum, and BRAF mutations occurred commonly in serrated lesions throughout the colon and rectum. This is different from the distribution of BRAF mutation observed in colorectal carcinomas. If only CRC data were evaluated, it would appear that BRAF mutation and CIMP are both specific to the right colon. However, our results suggest that the left and right sides of the colon are both susceptible to BRAF mutation but that the left colon and rectum are less susceptible than the right colon to CIMP and MLH1 methylation. A recent CRC study by Yamauchi and colleagues supports a gradient of increasing CIMP in tumors moving from the rectum to the right-colon; however, CIMP prevalence decreased in the cecum (43). Our serrated lesion results also suggest a gradient of increasing CIMP prevalence from the rectum to the right-colon, but unlike the Yamauchi study, this gradient continued to increase through the cecum.

**Prevalence of molecular markers by polyp size**

In addition to SSP histology and right-sided colon location, we reported that large serrated lesions (≥10 mm in diameter) had a high prevalence of all 3 molecular markers. This finding suggests that large serrated lesions may also be important candidates to determine CRC surveillance intervals. Several prior studies support this thesis, including a recent cross-sectional study reporting a 4.0-fold increase (95% CI, 2.8–5.7) in the odds of advanced colorectal neoplasia and a 4.8-fold increase (95% CI, 2.5–8.4) in the odds of proximal colon cancer associated with large serrated lesions (n = 140; ref. 44). Also, a separate study by Schreiner and colleagues reported a statistically significant association between large, right-sided serrated lesions (n = 44) and concurrent, as well as subsequent, colorectal neoplasia (45).

**Risk factors for CIMP-high and BRAF-mutated serrated lesions**

Although this is the first study to evaluate risk factors for CIMP and BRAF mutation in serrated lesions, prior studies have evaluated risk factors for CIMP-high and BRAF-mutated CRCs. Most of these prior studies do not present results for a direct comparison between subsets of CRC cases; thus, results from prior CRC studies should be interpreted with caution. However, a review of prior studies that evaluate risk factors associated with molecular subsets of CRC is still informative.

A recent cohort study, including more than 37,000 participants in the Iowa Women’s Study, reported a 1.9-fold (95% CI, 1.2–2.9) increase in the risk of CIMP-high CRC and a similar increase in the risk of BRAF-mutated CRC associated with current cigarette smoking but no association between smoking and CIMP-low/negative or wild-type BRAF CRC (25). These results were consistent with a prior report by Samowitz and colleagues (24). Similarly, we reported a statistically significant increase in the odds of CIMP-high and mutant BRAF serrated lesions associated with current smoking status. However, CIMP-low/negative and BRAF-wild-type serrated lesions also had strong, positive associations with current smoking status. These results suggest that smoking may act to initiate serrated lesions, through mechanisms that may be independent of BRAF mutation and CIMP.
Table 3. Polytomous logistic regression analysis of risk factors for serrated lesions by CIMP and *BRAF* mutation status clustered by participant: Group Health Enrollees 1998–2007

<table>
<thead>
<tr>
<th>Serrated lesions(^a) by CIMP status</th>
<th>Serrated lesions(^a) by <em>BRAF</em> mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls ((n = 1,027))</td>
<td>Wild-type <em>BRAF</em> ((n = 187)) Mutant <em>BRAF</em> ((n = 232))</td>
</tr>
<tr>
<td></td>
<td>% OR(^b) (95% CI) % OR(^b) (95% CI) (P_{\text{heterogeneity}}) % OR(^b) (95% CI) % OR(^b) (95% CI) (P_{\text{heterogeneity}})</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>na</td>
</tr>
<tr>
<td>Men</td>
<td>40</td>
</tr>
<tr>
<td>Caucasian race</td>
<td>86</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>15</td>
</tr>
<tr>
<td>Some college</td>
<td>25</td>
</tr>
<tr>
<td>College graduate</td>
<td>60</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>&lt;25</td>
</tr>
<tr>
<td></td>
<td>25–29</td>
</tr>
<tr>
<td></td>
<td>(\geq 30)</td>
</tr>
<tr>
<td>Regular NSAID use</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>Ever</td>
</tr>
<tr>
<td></td>
<td>CRC family history</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>Former</td>
</tr>
<tr>
<td></td>
<td>Current</td>
</tr>
<tr>
<td>Prior polyps</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^a\)419 serrated lesions among 359 participant clusters.

\(^b\)Adjusted for all other factors in Table 2 and study phase.

\(^c\)Wald test \(P\) value comparing polyp case subtypes with one another.
In addition to cigarette smoking, our results suggested a positive association between high BMI and serrated lesions regardless of CIMP and BRAF mutation status. Results from prior studies evaluating the association between BMI and subsets of CRC, by CIMP and/or BRAF mutation status are mixed. In a Netherlands cohort study, including more than 120,000 men and women, Hughes and colleagues reported positive associations between high BMI and both CIMP-high (n = 117) and CIMP-low/negative colorectal carcinomas (n = 318; ref. 23); however, only the association between CIMP-high CRC and BMI was statistically significant (HR comparing fourth quartile of BMI with first quartile = 1.77; 95% CI, 1.01–3.11). In contrast with these results, a case–control study including 783 disease-free controls, 130 CIMP-high, and 324 CIMP-low/negative CRC cases reported a significant association between BMI ≥ 30 kg/m² and CIMP-low/negative CRC (OR, 2.0; 95% CI, 1.5–2.6) and no association between BMI ≥ 30 kg/m² and CIMP-high (OR, 1.1; 95% CI, 0.8–1.7) or BRAF-mutated CRC (OR, 1.2; 95% CI, 0.6–2.1; ref. 46). The differences in results between these 2 studies may be due to chance variation, different study designs, statistical models, or CIMP panels.

Ethnicity and genetic variation have also been evaluated in relation to molecular subsets of CRC. In a cohort study of more than 41,000 participants from Australia, English and colleagues, compared those with Southern European ancestry to those with Anglo-Celtic ancestry and reported a lower incidence of CIMP-high [relative risk (RR), 0.32; 95% CI, 0.16–0.67] and mutant BRAF (RR, 0.30; 95% CI, 0.16–0.58) CRC associated with Southern European ancestry, but no association between ancestry and CIMP-low/negative or BRAF-wild-type CRC (22). Results from other studies suggest that certain genotypes may be specifically associated with the risk of CIMP-high CRC (47, 48). Thus, our results suggesting a positive association between CIMP-high serrated lesions and Caucasian race may potentially be mediated by genotypic variation between different racial and ethnic groups.

Limitations

Ours is the largest study of CIMP, BRAF mutation, and MLH1 methylation in colorectal adenomas and serrated lesions to date, but we had limited power to detect statistically significant variation in some risk factors between serrated lesion subtypes according to molecular characteristics. Reassuringly, analyses comparing serrated lesions subtypes to controls were well-powered and yielded results similar to prior studies evaluating molecular subsets of CRC. Also, colonoscopy-confirmed, polyp-free controls may introduce bias, because this group may be different than the underlying population with respect to health-seeking behaviors, perceived cancer risk, and other factors. However, because colorectal polyps are usually occult, using a colonoscopy-confirmed, polyp-free control group helped to limit the misclassification of disease status among controls. We were unable to compare our conventional adenoma results with previous studies using the same CIMP panel because other studies have not used this panel in conventional adenomas. Our decision to use the Weisenberger panel was driven by prior studies showing a strong correlation between this CIMP panel and BRAF mutation status in CRC (16, 31). However, it should be noted that validation analyses of CIMP panels specifically for colorectal polyps have not been conducted, and additional studies using the Weisenberger panel in a separate study population are needed to test the reproducibility of our results. Finally, there is significant inter-observer variability between pathologists in the classification of serrated lesions into specific subsets (49). To address this concern, our study pathologists used a training set of slides to standardize polyp classification, reviewed polyps in tandem, and reconciled disagreements with an additional slide review. Even with these quality control procedures, some misclassification of polyp histologic subtype may still occur.

Conclusions

On the basis of our study results and the results of prior studies, it is clear that molecular markers associated with the serrated pathway to CRC tend to co-occur in specific subsets of serrated lesions, specifically SSPs and large, right-sided serrated lesions. Furthermore, small longitudinal and cross-sectional epidemiologic studies support an increased risk of advanced colorectal neoplasia and cancer associated with these same subsets of colorectal polyps (41, 42, 44, 45). Also, for the first time, we have presented data linking risk factors for CIMP-high and BRAF-mutated serrated lesions to previously described risk factors for CRCs with these molecular markers. Large longitudinal studies are needed to characterize the risk of progression associated with subsets of serrated lesions and with the molecular markers they harbor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: A.N. Burnett-Hartman, P.A. Newcomb, K.W. Makar

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.N. Burnett-Hartman, P.A. Newcomb, J.D. Potter, L.C. Zhu, M.P. Upton, K.W. Makar

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.N. Burnett-Hartman, P.A. Newcomb, A.J. Pileps, M.P. Upton, K.W. Makar

Writing, review, and/or revision of the manuscript: A.N. Burnett-Hartman, P.A. Newcomb, J.D. Potter, M.N. Passarelli, A.J. Pileps, M.A. Wurscher, W.M. Grady, M.P. Upton, K.W. Makar

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.A. Newcomb, M.A. Wurscher

Study supervision: P.A. Newcomb, J.D. Potter, K.W. Makar

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