Evaluation of a Large, Population-Based Sample Supports a CpG Island Methylator Phenotype in Colon Cancer

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Background & Aims: The concept of a CpG island methylator phenotype (CIMP), especially in microsatellite stable colon cancer, is not accepted universally. We therefore evaluated a large population-based sample of individuals with colon cancer and used univariate and multivariate analyses of CIMP with clinicopathologic variables and tumor mutations to determine the biologic relevance of this phenotype. *Methods:* A total of 864 tumors from individuals with colon cancer from Utah and Northern California were evaluated by methylation-specific polymerase chain reaction of CpG islands in *hMLH1*, methylated in tumors (MINT) 1, MINT 2, MINT 31, and *CDKN2A (p16)*. CIMP high was defined as methylation at 2 or more of these loci. The *BRAF* V600E mutation was determined by sequencing. Microsatellite instability had been determined previously. *Results:* In a multivariate analysis of microsatellite stable tumors, CIMP high was related significantly to the V600E *BRAF* mutation (odds ratio, 39.52; 95% confidence interval, 11.44–136.56), *KRAS2* mutations (odds ratio, 2.22; 95% confidence interval, 1.48–3.34), older age (*P* trend = .03), and increased stage (*P* trend = .03), and these tumors were less likely to be located in the distal colon (odds ratio, .42; 95% confidence interval, .27–.65). CIMP-high unstable tumors also were more likely to have the V600E *BRAF* mutation, be located proximally, and occur in older individuals (in univariate analyses). However, CIMP-high unstable tumors were significantly more likely than their stable counterparts to be *KRAS2* wild type, *TP53* wild type, poorly differentiated, proximally located, occur at lower stages, and have the *BRAF* V600E mutation (64.1% vs 17.6%). *Conclusions:* The evaluation of a large, population-based sample strongly supports the biologic relevance of CIMP in colon cancer. However, the presence or absence of microsatellite instability has a major effect on the expression of this phenotype.

The concept of a CpG island methylator phenotype (CIMP) has had a complicated and somewhat controversial history. CIMP refers to the notion that a subset of tumors has widespread methylation of CpG islands that leads to epigenetic inactivation of tumor suppressor genes by promoter methylation. The original studies of 88 individuals with colorectal cancer reported CIMP in approximately 50% of colon cancers and noted significant relationships with proximal location, mutant *KRAS2*, and wild type *TP53*, relationships that were reported to be independent of microsatellite instability.[1,2] Two subsequent studies of relatively larger numbers of unselected colorectal cancer patients reported less frequent widespread methylation of CpG islands, especially if microsatellite unstable tumors were excluded, ranging from 12% to 25%.[3,4] Hawkins et al,[3] in a study of 396 individuals, reported relationships between CIMP and proximal location, female sex, older age, high tumor grade, mucinous histology, wild-type *TP53*, microsatellite instability, and mutant *KRAS2*. However, if microsatellite unstable tumors were excluded, significant relationships were seen only with older age, proximal location, mucinous histology, and mutant *KRAS2*. Rijnsoever et al,[4] in a study of 275 individuals, reported relationships between CIMP and poor differentiation, wild-type *TP53*, proximal location, and higher stage, with or without inclusion of microsatellite unstable tumors. Recent studies also have identified an excess of the *BRAF* V600E mutation in CIMP-high stable and unstable tumors.[5,6]

Microsatellite instability by itself has been associated with an inverse relationship with *KRAS2* and *TP53* mutations, a better prognosis than stable tumors, and proximal tumor location.[7,8] The majority of sporadic microsatellite

Abbreviations used in this paper: AJCC, American Joint Committee on Cancer; CI, confidence interval; CIMP, CpG island methylator phenotype; HNPCC, hereditary nonpolyposis colon cancer; MINT, methylated in tumors; OR, odds ratio; TGF-β RII, transforming growth factor β receptor type II.

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unstable tumors also are methylated heavily,[1,2] and it therefore may be important to consider the CIMP status of tumors with and without microsatellite instability separately to ascertain the true contribution of CIMP to these various relationships. The relationship of CIMP to family history also is controversial, with 1 study reporting a relationship between CIMP and family history of cancer yet another study showed no such relationship.[9,10] Finally, the entire notion of CIMP recently has been challenged, with the assertion that the division of CIMP-negative and CIMP-positive tumors is arbitrary and that without inclusion of microsatellite unstable cancers most of the reported relationships with CIMP, other than age and proximal location, disappear.[11]

It should be noted that although some of the previous studies of CIMP were unselected, none were population based. Also, none of the previous studies performed multivariate analyses of CIMP and its relationships or had sufficient power to compare adequately CIMP-high stable with CIMP-high unstable carcinomas. In this study we have determined the significance of CIMP with and without microsatellite instability. We also compare CIMP-high stable and unstable carcinomas and perform multivariate analyses of CIMP and its clinicopathologic relationships to determine whether relationships independent of age and/or proximal location exist in stable tumors.

Materials and Methods

Study Population

Study participants were white, black, or Hispanic and were from either the Kaiser Permanente Medical Care Program of Northern California or an 8-county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties). Eligibility criteria for inclusion in the study included diagnosis with first primary incident colon cancer (International Classification of Diseases, 9th revision, 2nd edition codes 18.0 and 18.2–18.9) between October 1, 1991, and September 30, 1994, age between 30 and 79 years at the time of diagnosis, and mentally competent to participate in the study. Patients with cancer of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening) or with known familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were not eligible. All cases were adenocarcinomas or carcinomas. This study population is part of a previously described sample.[12] Tumor blocks and amplifiable DNA originally were available for 1530 individuals; this represents 84% of all individuals diagnosed with colon cancer, making this a truly population-based sample. This sample has been used for previous population-based studies on *KRAS2*, *TP53*, and microsatellite instability.[7,13,14] Sufficient DNA for determination of CIMP (which requires a fairly large aliquot of DNA) was available for tumors from 864 individuals. This group did not differ from those for whom CIMP was not determined with respect to age, American Joint Committee on Cancer (AJCC) stage, histologic differentiation, tumor site, prognosis, or family history of colorectal cancer (data available on request).

Information on age at time of diagnosis, sex, tumor site, and tumor stage was available from the Northern California Tumor Registry, the Sacramento Tumor Registry, and the Utah Cancer Registry. These registries are members of the Surveillance, Epidemiology, and End Results program. Tumors occurring in the cecum through the transverse colon were defined as proximal; tumors in the splenic flexure, descending, and sigmoid colon were defined as distal. Tumors were staged according to AJCC[15] criteria and the histologic grade and presence or absence of mucinous histology was determined by reviewing pathology reports. Because we did not have access to complete medical records, AJCC stage IV tumors were identified by using Surveillance, Epidemiology, and End Results program summary stage codes to determine whether distant metastases were present. All aspects of this study were approved by the University of Utah and Kaiser Permanente Medical Care Program institutional review boards.

CIMP

Sodium bisulfate modification was performed on DNA extracted from tumors microdissected for previous studies.[7] Methylation-specific polymerase chain reaction then was performed as described previously for the following CpG islands: methylated in tumors (MINT) 1, MINT 2, MINT 31, *CDKN2A(p16)*, and *hMLH1*.[16] This panel was being used at the time our study began by the group who originally described CIMP and its importance in colorectal cancer, and their criterion for CIMP high was methylation of 2 or more of these CpG islands.[16] Methylation was defined as a recognizable band on an agarose gel by using the methylation-specific primers. CIMP low was defined as less than 2 of 5 markers methylated. The primers used for *hMLH1* methylation as part of the CIMP panel are located approximately 170 and 270 base pairs 5′ of the start codon. We also determined *hMLH1* methylation by using a different set of primers located approximately 650–800 base pairs 5′ to the start codon,[17] but this result was not used for the determination of CIMP high and low.

BRAF V600E Mutation Detection

The *BRAF* V600E mutation was detected by amplifying exon 15 of *BRAF* by using the forward primer 5′-TCA TAA TGC TTG CTC TGA TAG GA-3′ and the reverse primer 5′-CTT TCT AGT AAC TCA GCA GC-3′. Amplifications were performed using AmpliTaq Gold (Applied Biosystems, Foster City, CA) and a polymerase chain reaction profile consisting of a 9-minute initial denaturation at 95°C, then 35 cycles of 20 seconds at 95°C, 20 seconds at 60°C, and 30 seconds at 72°C, with a 5-minute final extension at 72°C. Mutations were verified by sequencing in both directions.