Commentary

The CpG Island Methylator Phenotype in Colorectal Cancer

Wade S. Samowitz

From the University of Utah Health Sciences Center, Department of Anatomic Pathology, Salt Lake City, Utah

The concept of a CpG island methylator phenotype, or 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.¹ Perhaps the best accepted example of this is sporadic colorectal tumors with microsatellite instability. These tumors exhibit extensive CpG island methylation, and their instability, in most cases, is due to promoter methylation of one of the DNA mismatch repair genes, *hMLH1*.² In contrast, microsatellite instability in tumors associated with hereditary non-polyposis colorectal cancer (HNPCC) is due to a germline mutation in one of the mismatch repair genes. The concept of CIMP has not, however, been universally accepted, especially with respect to colorectal tumors without microsatellite instability.³

The study by Ogino et al⁴ in the current issue of *The Journal of Molecular Diagnostics* represents a significant advance in the field of CIMP. This study of over 900 colorectal tumors uses a quantitative method for characterization of CpG island methylation status and evaluates multiple panels of CpG islands for the classification of CIMP. Although questions remain, a strong implication of this study is that this method may be used to help determine which microsatellite unstable tumors are sporadic and which are associated with HNPCC. In this Commentary, we will give a brief background of CIMP, discuss the contribution of the Ogino et al study,⁴ and then detail unanswered questions and future directions for research.

Background

CIMP was originally described in colorectal cancer by Toyota et al in 1999.¹ This and subsequent studies identified a number of relationships between CIMP and various clinicopathological parameters, including microsatellite instability, proximal location, mutant *Ki-ras*, wild-type *p53*, female gender, older age, and, especially, the BRAF

V600E mutation.^{1,5-9} The concept of CIMP was challenged, however, since it was asserted that the division of CIMP-negative and CIMP-positive tumors was arbitrary and that without inclusion of microsatellite unstable tumors, most of the reported relationships, other than older age and proximal location, would disappear.³ Our multivariate analysis of colon tumors showed that this was not the case, since significant relationships were seen in the subset of tumors without microsatellite instability and with factors besides age and location, including the BRAF V600E mutation, *Ki-ras* mutations, and higher stage.² However, a bimodal distribution of microsatellite stable colon cancers with respect to CIMP was not seen.

Many of these studies, including our own, used the so-called classic panel of CpG islands: *hMLH1*, *p16*, and MINTS (methylated in tumors)¹ 1, 2, and 31, and also used methylation-specific polymerase chain reaction (PCR)¹⁰ to determine the methylation status of the respective CpG island. The next major development was by Weisenberger et al.¹¹ They used MethyLight technology and an unsupervised two-dimensional cluster analysis of DNA methylation data to identify CpG island panels in which CIMP classification showed a very strong relationship to BRAF V600E mutations, seemed to be associated with nearly every sporadic microsatellite unstable tumor, and, for the first time, showed a bimodal distribution with respect to colorectal cancers, implying that the distinction between CIMP-positive and -negative was not arbitrary (as had been previously suggested³). A five-marker CpG island panel (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOC1*) was identified that demonstrated both bimodality and robust performance on formalin-fixed, paraffin-embedded tissue.

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Address reprint requests to Wade S. Samowitz, M.D., University of Utah Health Sciences Center, Department of Pathology, 50 North Medical Dr., Salt Lake City, UT 84132. E-mail: wade.samowitz@aruplab.com.

The Current Study

The study by Ogino et al⁴ takes the necessary step of validating the CpG island panels and methods proposed by Weisenberger et al¹¹ in a different and larger set of colorectal tumors. This elegant study compares several modifications of the panels initially proposed by Weisenberger et al,¹¹ shows that they are essentially identical in their classification of CIMP, and even defines a slightly different and, perhaps more manageable, panel of four CpG islands (*RUNX3*, *CACNA1G*, *IGF2*, and *hMLH1*) that demonstrate very high sensitivity and specificity for CIMP. The excellent bimodal distribution of CIMP in microsatellite unstable tumors demonstrated by Ogino et al⁴ suggests that this approach may prove useful for the diagnostic work-up of HNPCC, since CIMP-high microsatellite unstable tumors are very likely to be sporadic.

As might be expected, however, some differences with Weisenberger et al¹¹ were noted in this larger sample. The original study stated that with one exception all microsatellite unstable cancers could be explained by either an HNPCC germline mutation or by CIMP-high associated with *hMLH1* methylation. In the current study, close to 20% of microsatellite unstable tumors were CIMP-negative; although Ogino et al⁴ have not evaluated the individuals with microsatellite unstable tumors for germline mismatch repair gene mutations, they did conclude that it is unlikely that all of these 20% are due to HNPCC. We have observed a similar group of apparently non-HNPCC, CIMP-negative tumors in our dataset,² and Oliveira et al¹² observed that approximately 40% of sporadic unstable tumors lacked *hMLH1* methylation. If true, this has important implications for the interpretation of CIMP in the context of the work-up for HNPCC. Whereas CIMP-high, microsatellite unstable tumors may be reliably regarded as sporadic, it seems imprudent at the present time to regard all microsatellite unstable, CIMP-negative tumors as necessarily HNPCC-associated. This is similar to the situation with *BRAF* V600E mutations in microsatellite unstable cancers: the presence of a mutation is strong evidence that the tumor is sporadic, whereas the absence of the mutation suggests that the tumor may be either sporadic or HNPCC-associated.^{13–15}

Another difference is that the relationship between CIMP and *BRAF* V600E mutations, at least in tumors without microsatellite instability, appears not to be as strong in the study by Ogino et al⁴ (using their criteria for CIMP-high) as that in Weisenberger et al.¹¹ In the previous study, only two of 26 cancers (8%) with *BRAF* mutations occurred in tumors that were not CIMP-high¹⁰ (although this analysis did include microsatellite unstable cancers). In the current study, depending on the cut-off used for CIMP-high, approximately 40 to 60% of microsatellite stable tumors with *BRAF* mutations occurred in tumors that were not CIMP-high. It should be noted that this also does not compare well with our previous results using the classic CpG island panel and methylation-specific PCR, as in that study only three of 35 (9%) microsatellite stable cancers with *BRAF* mutations occurred in tumors that were not CIMP-high.²

Finally, although Ogino et al⁴ demonstrate a very impressive (and, probably, diagnostically useful) bimodal

distribution for CIMP in microsatellite unstable tumors, no such bimodal distribution is seen for microsatellite stable tumors. This is very similar to our results using the classic panel of CpG islands and methylation-specific PCR, although the bimodal distribution for unstable tumors in our dataset is not as distinct.² This result of Ogino et al⁴ may not even be a difference from that originally reported by Weisenberger et al,¹¹ since that study did not separately report distributions for microsatellite stable and unstable cancers. At any rate, the lack of a bimodal distribution of CIMP, and therefore the arbitrary designation of this parameter, if unstable tumors are excluded was one of the original criticisms of the concept of CIMP; although we² have shown important relationships between CIMP in stable tumors and a variety of clinicopathologic variables, especially *BRAF* mutations, this remains an unresolved issue.

Future Directions

Although Ogino et al⁴ have made great strides in describing a panel and technique for CIMP determination that will probably have important diagnostic implications, there are still unanswered questions regarding CIMP. One is which technique is the best for determination of methylation. Whereas Weisenberger et al¹¹ and Ogino et al⁴ make a strong case for MethyLight, other techniques have been used, including methylation-specific PCR and pyrosequencing.¹⁶ We are unaware of a study that directly compares these techniques, although such studies may be underway.

The main unresolved issue with CIMP is which panel is the best for CIMP determination, or whether even one panel will suffice. The major problem with this is that there is no gold standard for CIMP; both Ogino et al⁴ and Weisenberger et al¹¹ use surrogate markers (such as relationships to *BRAF* and/or *Ki-ras* mutations or microsatellite instability) as a way to argue that a particular panel is better than another. Although no other obvious method is available at this time, there are suggestions that this does not necessarily result in an optimal panel, at least for all subsets of tumors. We would argue that the strongest results using these panels are for microsatellite unstable tumors, where there is an obvious bimodal distribution and very strong relationships with *BRAF* and *Ki-ras* mutations (direct and inverse, respectively). The situation is not so clear for microsatellite stable tumors, however. As noted above, the classic panel was much more sensitive for *BRAF* mutations in microsatellite stable tumors in our dataset² as compared with Ogino et al.⁴ In addition, in a recent study that related CIMP to cigarette smoking, we identified a significant relationship between smoking and CIMP-high, *BRAF* wild-type tumors,¹⁷ a relationship that might not have been seen if we had used a definition of CIMP that was more specific for *BRAF* mutations (as in Weisenberger et al¹¹ or Ogino et al⁴). There has been a suggestion in the literature that three classifications of CIMP exist: CIMP-negative, CIMP-low, and CIMP-high. We have noted that *BRAF* mutations are associated with very high degrees of methylation, and

Ogino et al⁴ have previously proposed that a CIMP-low classification may exist that is related to *Ki-ras* mutations rather than *BRAF*.^{17,18} The classic CIMP panel, precisely because it is not as tightly linked to *BRAF* mutations as those proposed by Ogino et al,⁴ shows direct relationships with both *Ki-ras* and *BRAF*, mutations that are mutually exclusive because they occur in the same pathway.¹⁹ This suggests that the classic panel is combining, to a certain extent, the CIMP-low and CIMP-high categories. This may be more useful for epidemiological studies than the new proposed panels. At any rate, we do not feel that the optimal panel or panels for CIMP have been as yet identified, and future research should try to determine such panels but also take this uncertainty into account.

In summary, this elegant study by Ogino et al⁴ performs an essential validation of the findings of Weisenberger et al¹¹ and also points to some differences with the previous study that may have implications for the interpretation of CIMP results in the work-up of HNPCC. Much work still needs to be done with respect to the optimal technique(s) and panel(s) for CIMP determination, but the diagnostic utility and epidemiology of this epigenetic change strongly support its significance in the biology of colorectal cancer.

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