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Perspective

Cancer Research

The CpG Island Methylator Phenotype: What's in a Name?

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Abstract

Although the CpG island methylator phenotype (CIMP) was first identified and has been most extensively studied in colorectal cancer, the term "CIMP" has been repeatedly used over the past decade to describe CpG island promoter methylation in other tumor types, including bladder, breast, endometrial, gastric, glioblastoma (gliomas), hepatocellular, lung, ovarian, pancreatic, renal cell, and prostate cancers, as well as for leukemia, melanoma, duodenal adenocarninomas, adrenocortical carcinomas, and neuroblastomas. CIMP has been reported to be useful for predicting prognosis and response to treatment in a variety of tumor types, but it remains unclear whether or not CIMP is a universal phenomenon across human neoplasia or if there should be cancer-specific definitions of the phenotype. Recently, it was shown that somatic *isocitrate dehydrogenase-1* (*IDH1*) mutations, frequently observed in gliomas, establish CIMP in primary human astrocytes by remodeling the methylome. Interestingly, somatic *IDH1* and *IDH2* mutations, and loss-of-function mutations in ten-eleven translocation (*TET*) *methylcytosine dioxygenase-2* (*TET2*) associated with a hypermethylation phenotype, are also found in multiple enchondromas of patients with Ollier disease and Mafucci syndrome, and leukemia, respectively. These data provide the first clues for the elucidation of a molecular basis for CIMP. Although CIMP appears as a phenomenon that occurs in various cancer types, the definition is poorly defined and differs for each tumor. The current perspective discusses the use of the term CIMP in cancer, its significance in clinical practice, and future directions that may aid in identifying the true cause and definition of CIMP in different forms of human neoplasia. *Cancer Res; 1–11. ©2013 AACR.*

Introduction

Unraveling the complexities of the epigenetic code has been instrumental in advancing our understanding of cancer etiology. It is now clear that epigenetic modifications including aberrant DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs play a significant role in cancer development (1). Because such processes do not induce changes in the DNA sequence, but rather are self-propagating molecular signatures that are potentially reversible (2, 3), they provide novel targets for diagnosis and treatment strategies (1, 4, 5).

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DNA hypermethylation of promoter-associated CpG islands of tumor suppressor and DNA repair genes, which leads to transcriptional silencing of these genes, has been the most studied epigenetic alteration in human neoplasia (1). Widespread CpG island promoter methylation, also referred to as the CpG island methylator phenotype (CIMP), was first identified (6) and has been extensively studied in colorectal cancer. Recently, we systematically reviewed the body of colorectal cancer CIMP research and concluded that because there is no universal standard or consensus with respect to defining CIMP, establishing the true prevalence of CIMP in colorectal cancer will be challenging until its biologic cause is determined (7).

Despite these limitations identified in colorectal cancer research, the term "CIMP" has been repeatedly used over the past decade to describe the increased prevalence of CpG island promoter methylation in other tumor types, including bladder (8), breast (9–11), endometrial (12, 13), gastric (14–19), glioblastoma (gliomas; refs. 20–22), hepatocellular (23–26), lung (27, 28), ovarian (29), pancreatic (30), prostate (31), and renal cell (32) cancers, as well as in leukemia (33–36), melanoma (37), duodenal adenocarninomas (38), adrenocortical carcinomas (39), and neuroblastomas (40, 41). The primary purpose of these studies was to determine if CIMP is also present in these cancers, and if it can be used to distinguish between known phenotypes of the respective cancer type. However, in many cases, the observation of CIMP for a tumor results from a self-fulfilling definition, where a subgroup of tumors with a greater

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degree of DNA methylation than the remaining tumors constitutes CIMP.

Although CIMP has been associated with environmental and lifestyle factors (3, 42–48), the molecular basis for CIMP is only beginning to be explored. The first clues came from two studies showing that glioblastomas with a hypermethylator phenotype are associated with somatic mutations in *isocitrate dehydrogenase-1* (*IDH1*; refs. 20, 21), and that somatic mutations in *IDH1*, *IDH2,* as well as loss-of-function mutations in ten-eleven translocation *(TET)-methylcytosine dioxygenase-2* (*TET2)* establish a hypermethylation phenotype in leukemia (49). These are the first indications for a molecular basis of CIMP, and provide an explanation for a very distinct set of tumors with increased levels of hypermethylated DNA. Consequently, these studies have provided a framework for understanding the interplay between genetic and epigenetic changes, and also raise questions about the causes and importance of CIMP in other tumor types. Is "CIMP" a universal phenomenon across human neoplasia caused by similar defects and characterized by similar hypermethylomes, or are there tumor type–specific causes and tumor type–specific definitions of the phenotype?

Addressing these questions is essential for directing research at exploiting CIMP. Here, we discuss the evolution in our understanding of CIMP in various tumor types and how the recent characterization of the human cancer genome and epigenome may influence future research.

CIMP: Roots in Colorectal Cancer

Molecular characteristics of CIMP tumors

Before any discussion on CIMP, it is important to briefly describe CIMP in colorectal cancer, as much of the research surrounding CIMP in other cancer types is based on this body of evidence. It has been more than a decade since Toyota and colleagues first identified CIMP in colorectal cancer (6). Colorectal cancer tumors characterized by CIMP have distinctly different histology when compared with tumors derived from traditional adenoma-carcinoma pathway (50–53). An early event in CIMP tumors seems to be the ^{V600E}*BRAF* mutation (53). A tight association between the ^{V600E}*BRAF* mutation and CIMP, and mice data showing that the ^{V600E}*BRAF* mutation in the mouse gut induces increased DNMT3B expression, *de novo* methylation, and downregulation of specific CpG dinucleotides in $p16^{INK4A}$ exon 1, has been reported (54). However, there is no functional evidence supporting that the ^{V600E}*BRAF* mutation is a causal event for CIMP. Therefore, it remains possible that *BRAF* mutation is a surrogate marker for another causal gene. Furthermore, most CIMP colorectal cancers are characterized by promoter CpG island hypermethylation of the mismatch repair gene, *MLH1*, resulting in its transcriptional inactivation. Loss of *MLH1* is thought to cause microsatellite instability (MSI), a form of genetic instability characterized by length alterations within simple repeated microsatellite sequences of DNA (51, 55). Once *MLH1* is inactivated, the rate of progression to malignant transformation is rapid (53).

In 2006, a major advancement was made in CIMP research by using unsupervised hierarchical cluster analysis of methylation data; Weisenberger and colleagues identified a robust 5-gene panel that recognized a distinct, heavily methylated subset of colorectal tumors that were also characterized by the ^{V600E}*BRAF* mutation and MSI (56). This panel proved the validity of the phenotype in colorectal cancer, which has been further substantiated and validated in a large, population-based sample (57). Since then, the combinations of genes in addition to those proposed by the Weisenberger and colleagues have been suggested as the "best" panel (58–61), but the idea that CIMP is tightly linked with the ^{V600E}*BRAF* mutation remains consistent in all studies. However, a cause or molecular mechanism for CIMP in colorectal cancer has not yet been identified, and thus the sensitivity and specificity of this panel for defining CIMP remains to be established. Another aspect that needs to be resolved is the question of whether colorectal cancer CIMP cases should be further subgrouped in CIMP-high and CIMP-low colorectal cancers (58–60, 62–64). Although CIMP-low colorectal cancers have been associated with KRAS mutations, this group has many clinical and pathologic features in common with non-CIMP, and consensus on how to define CIMP-low is currently lacking.

CIMP translated to other cancer types

From the literature, it is evident that many studies have investigated CIMP on the premise that the phenotype and genes that quantify the phenotype are not cancer type specific, but rather universal. For example, studies involving breast and endometrial cancer have defined CIMP as "methylated multigenes in tumors" (11) and "when multiple genes are concurrently methylated" (13), respectively. The definition of "multiple" is defined by each investigator to provide separations into subgroups of patients. Furthermore, it is not uncommon for researchers investigating tumor types other than colorectal cancer to refer the study of Weisenberger and colleagues (56) as a rationale for studying CIMP as a marker of cancer, even though the results of that study were very specific for colorectal cancer, especially for tumors characterized by the ^{V600E}*BRAF* mutation.

In our recent review, we detailed the use of various techniques and multiple gene panels and cutoff thresholds used to classify a colorectal cancer tumor as CIMP-positive (7). Selection of gene panels and cutoff thresholds for defining CIMP and small sample sizes in other tumor types seems to be even more arbitrary than for colorectal cancer (Table 1). Studies in gastric cancer (14–19) have often been based on the "classic" gene panel first identified in colorectal cancer by Toyota and colleagues (6), before Weisenberger and colleagues (56). Studies in ovarian cancer (29), breast cancer (11), hepatocellular carcinoma (23, 26), and melanoma (37) have in part chosen gene panels based on observations from colorectal cancer or gastric cancer research. It is not our intention to imply that such studies are inherently flawed, but again, this type of selection assumes that CIMP is a universal process and not cancer specific.

Extensive studies of genetic and epigenetic changes in human cancers show that the transformation process differs greatly among tumors arising in different organs. Thus, if CIMP is ultimately organ or tissue specific, much of the true picture surrounding prevalence and prognostic value may not be

Table 1. Summary of studies of CIMP detection and status

	Study characteristics			Assessment of CIMP		
Study	Country	*N*	Gene panel[a]	Method	Marker threshold to assign CIMP-H	% CIMP-H[b]
Adrenocortical carcinomas						
Barreau and colleagues (39)	France	51	Genome-wide characterization of methylome and methylation-specific multiplex ligation-dependent probe of 33 genes identified in the genome-wide Infinium analysis	Infinium HumanMethylation27 arrays	Clustering analysis	16%
Bladder cancer						
Maruyama and colleagues (8)	United States	98	*CHD1*, *RASSF1A*, *APC*, *CDH13*, *FHIT*, *RARB (RARβ)*, *GSTP1*, *CDKN2A (p16)*, *DAPK1 (DAPK)*, *MGMT*	MSP	≥4/10 Genes methylated	16%
Breast cancer						
Bae and colleagues (9)	Korea/United States	109	*RASSF1A*, *SCGB3A1(HIN1)*, *TWIST1 (Twist)*, *CCND2 (cyclin D2)*, *RARB (RARβ)*, *THRB (THRβ)*, *CDH1 (E-cadherin)*, *ESR1 (ER)*, *BRCA1*, *GSTP1*, *BAX*, *RB1 (RB)*	MSP	–-[c]	Conclude that CIMP does not exist in breast cancer
Jing and colleagues (11)	China	50 Tumors	*RASSF1A*, *BRCA1*, *CDKN2A (p16)*, *CDH1*, *ESR1 (ER)*, *RARB (RARβ2)*, *PTGS2 (COX-2)*, *APC*, *DAPK1 (DAPK)*, *FHIT*	MSP	≥3/10 Genes methylated	78%
		50 Nontumor serum				9%
Fang and colleagues (10)	United States	39	Genome-wide characterization of methylome	EpiTYPER system (Sequenom)	Characterized by the presence or absence of coordinate hypermethylation at a large number of genes	44%
Endometrial cancer						
Whitcomb and colleagues (12)	United States	24	*HOXA11*, *THBS1*, *THBS2*, *CTNNB1*, *VDR*, *MLH1*, *CDKN2A*	COBRA	≥5/7 Genes methylated	"it exists"
Zhang and colleagues (13)	China	35	*CDKN2A (P14)*, *CDKN2A (P16)*, *ESR1 (ER)*, *PTGS2 (COX-2)*, *RASSF1A*	MSP	≥3/5 Genes methylated	49%
Duodenal adenocarcinoma						
Fu and colleagues (38)	United States	98	*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, *SOCS1*	MethyLight	≥3/5 Genes methylated	27%
Gastric cancer						
Toyota and colleagues (19)	United States	56	*MINT1*, *MINT2*, *MINT12*, *MINT25*, *MINT31*	MSP	≥3/5 Genes methylated	41%
Oue and colleagues (18)	Japan	103	*MINT1*, *MINT2*, *MINT12*, *MINT25*, *MINT31*	MSP	≥3/5 Genes methylated	41%
Kim and colleagues (16)	South Korea	79	*MINT1*, *MINT2*, *MINT12*, *MINT25*, *MINT31*	COBRA	≥3/5 Genes methylated	24%
Etoh and colleagues (15)	Japan	105	*CDKN2A (P16)*, *MLH1 (hMLH1)*, *THBS1 (THBS-1)*, *MINT1*, *MINT2*, *MINT12*, *MINT31*	MSP	≥3/7 Genes methylated	24%

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Table 1. Summary of studies of CIMP detection and status (Cont'd)

	Study characteristics			Assessment of CIMP		
Study	**Country**	***N***	**Gene panel[a]**	**Method**	**Marker threshold to assign CIMP-H**	**% CIMP-H[b]**
An and colleagues (14)	United States	83	*MINT1*, *MINT2*, *MINT25*, *MINT31*	MSP	≥2/4 Genes methylated	31%
Kusano and colleagues (17)	Japan	78	*MINT1*, *MINT2*, *MINT12*, *MINT25*, *MINT31*	COBRA	≥4/5 Genes methylated	24%
Gliomas						
Noushmehr and colleagues (20)		272	Genome-wide characterization of the methylome	Infinium+ Golden Gate methylation assays	Clustering analysis	9%
		208	*SOWAHA (ANKRD43)*, *HFE*, *MAL*, *LGALS3*, *FAS (FAS-1)*, *(FAS-2)*, *RHOF (RHO-F)*	MethyLight	DOCK5 hypomethylation + ≥5/7 genes methylated	8%
van den Bent and colleagues (22)	Europe (EORTC study 26951, the Netherlands	68	Genome-wide characterization of the methylome	Infinium HumanMethylation27 arrays	Clustering analysis + Noushmehr definition	46%
Hepatocellular cancer						
Shen and colleagues (25)	China, England, United States	85	*CDKN2A (p16)*, *CACNA1G*, *PTGS2 (COX-2)*, *ESR1 (ER)*, *MINT1*, *MINT2*, *MINT27*, *MINT31*	MSP	≥2/8 Genes methylated	38%
Zhang and colleagues (26)	China	50	*CDKN2A (P14)*, *CDKN2B (P15)*, *CDKN2A (P16)*, *TP53 (P53)*, *RB1*, *ESR1 (ER)*, *WT1 (WTI)*, *RASSF1A*, *MYC (c-Myc)*	MSP	≥5/8 Genes methylated	70%
Cheng and colleagues (23)	China	60	*CDKN2A (P14)*, *CDKN2B (P15)*, *CDKN2A (P16)*, *CDKN1A (P21)*, *SYK*, *TIMP3 (TIMP-3)*, *WT1*, *CDH1 (E-cadherin)*, *RASSF1A*, *RB1*	MSP	≥4/10 Genes methylated	32%
Leukemia						
Toyota and colleagues (36)	United States	36	*ESR1 (ER)*, *CACNA1G*, *MINT1*, *MINT2*, *CDKN2A (p16INK4A)*, *THBS1*, *CDKN2B (p15INK4B)*, *PTCH1 (PTC1A, PTC1B)*, *ABCB1 (MDR1)*, *MYOD1 (MYOD)*, *SDC4*, *GRP37*, *PITX2*, *MLH1*	Bisulfite-PCR	≥8/14 Genes methylated	19%
Garcia-Manero and colleagues (33)	United States	80	*ESR1 (ER)*, *CDKN2B (p15)*, *CDKN2A (p16)*, *ABCB1 (MDR1)*, *THBS1*, *THBS2*, *ABL1 (C-ABL)*, *TP73 (p73)*, *MYOD1 (MYF3)*, *MME (CD10)*	Bisulfite-PCR	≥3/10 Genes methylated	43%
Roman-Gomez and colleagues (35)	Spain	50	*ADAMTS1 (ADAMTS-1)*, *ADAMTS5 (ADAMTS-5)*, *APAF1 (APAF-1)*, *PPP1R1BB (ASPP-1)*, *CDH1*, *CDH13*, *DAPK1 (DAPK)*, *DIABLO*, *DKK3 (DKK-3)*, *LATS1 (LATS-1)*, *LATS2 (LATS-2)*, *KLK10 (NES-1)*, *CDKN2A (p14)*, *CDKN2B (p15)*, *CDKN2A (p16)*, *CDKN1C (p57)*, *TP73 (p73)*, *PARK2 (PARK-2)*, *PTEN*, *SFRP1/2/4/5 (sFRP1/2/4/5)*, *PTPN6 (SHP-1)*, *SYK*, *PYCARD (TMS-1)*, *WIF1 (WIF-1)*	MSP	≥3 Methylated genes	76%

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Table 1. Summary of studies of CIMP detection and status (Cont'd)

	Study characteristics			Assessment of CIMP		
Study	Country	*N*	Gene panel[a]	Method	Marker threshold to assign CIMP-H	% CIMP-H[b]
Roman-Gomez and colleagues (34)	Spain	54	38 Genes involved in cell immortalization and transformation	MSP	≥3 Methylated genes	63%
Figueroa and colleagues (49)	United States	385	Genome-wide characterization of the methylome	Roche Nimblegen custom human promoter array covering 25,626 *Hpa*II amplifiable fragments and MassArray Epityping	Clustering analyses	–
Lung cancer						
Suzuki and colleagues (28)	Japan	150	*TMEFF2 (HPP1)*, *SPARC*, *RPRM (Reprimo)*, *RBP1 (CRBP1)*, *RARB (RARβ)*, *RASSF1A*, *APC*, *CDH13*, *CDKN2A (p16^{INK4A})*	MSP	—	33%
Liu and colleagues (27)	China	60	*OGG1 (hOGG1)*, *VHL*, *RARB (RAR-B)*, *MLH1 (hMLH1)*, *SEMA3B*, *RASSF1A*, *ZMYND10 (BLU)*, *FHIT*	MSP	≥4/8 Genes methylated	57%
Melanoma						
Tanemura and colleagues (37)	United States	122	*WIF1*, *TFPI2*, *RASSF1A*, *RARB (RARβ2)*, *SOCS1*, *GATA4*, *MINT1*, *MINT2*, *MINT3*, *MINT12*, *MINT17*, *MINT25*, *MINT31*	MSP	—	—
Neuroblastoma						
Abe and colleagues (40)	Japan	140	17 Members of *PCDHB* family, 13 members of *PCDHA* family, *MST1 (HLP)*, *DKFZp451I127*, *CYP26C1*	qMSP	Cutoff >40% methylation of *PCDHB* family members	—
Abe and colleagues (41)	Germany	152	17 Members of *PCDHB* family, *MST1 (HLP)*, *CYP26C1*	qMSP	>60% Methylation of *PCDHB* family members and for samples with 40% to 60% *PCDHB* methylation, >10% *MST1 (HLP)* methylation and/or >70% *CYP26C1* methylation	33%
Ovarian cancer						
Strathdee and colleagues (29)	Scotland	93	*BRCA1*, *HIC1*, *MLH1*, *CDKN2A (p16)*, *TERC (hTR)*, *CASP8*, *MINT25*, *MINT31*, *CDKN2B (p15)*, *TP73 (p73)*	MSP	Unclear, although they do make a conclusion about CIMP	Unclear; 71% of tumors showed methylation
Pancreatic cancer						
Ueki and colleagues (30)	United States	45	*RARB (RARβ)*, *THBS1*, *CACNA1G*, *MLH1*, *MINT1*, *MINT2*, *MINT31*, *MINT32*	MSP	≥4/8 Genes methylated	14%
Prostate cancer						
Maruyama and colleagues (31)	United States	101	*RARB (RARβ)*, *RASSF1A*, *GSTP1*, *CDH13*, *APC*, *CDH1*, *FHIT*, *CDKN2A (p16^{INK4A})*, *DAPK1 (DAPK)*, *MGMT*	MSP	—	—

Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; MSP, methylation specific PCR analysis; qMSP, quantitative methylation specific PCR analysis.

[a]Gene names are reported as HUGO approved gene symbols, between brackets the gene symbols used in the original study.

[b]CIMP-H refers to either CIMP or in the instance that a study reported three CIMP categories, CIMP-high.

[c]Data not reported.

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recognized with the use of CIMP markers developed in another tumor type. For example, in a study of CIMP in endometrial cancer, genes were selected on the basis of their high degree of methylation in other malignancies, including colorectal cancer (13). However, a recent molecular characterization of endometrial tumors identified no ^{V600E}*BRAF* mutations in any of the 87 specimens considered (65). Therefore, selecting a CIMP panel tightly associated with *BRAF* mutation may not be entirely relevant to quantifying or identifying CIMP in endometrial tumors. Similarly, results from a recent study on duodenal adenocarcinomas suggest that *BRAF* mutations are not involved in duodenal tumorigenesis, MSI, or CIMP development (38). If one hypothesizes that CIMP is a general phenomenon, then the cause of CIMP should also be general and similar across different cancer types.

To assess just how universal CIMP is across tumor types requires genome-wide characterization of the methylome. This is a relatively new direction in epigenetic research, and to our knowledge, has only been reported for gliomas (20), leukemia (49), breast cancer (10), benign nonhereditary skeletal tumors such as enchondroma (66), as well as, most recently, renal cell carcinoma (32), melanoma (67), gastric cancer (68), and oral squamous cell carcinoma (69).

CIMP: Genome-Wide Characterization of the Methylome

Glioma

Promoter-associated hypermethylation has been commonly reported in gliomas (70–76), but it was not until 2010, when Noushmehr and colleagues used Ilumina array platform technology, that a CIMP specific for a group of gliomas with distinct molecular and clinical characteristics was established (20). They referred to this cluster of tumors as "G-CIMP" to imply its specificity for this tumor type. G-CIMP loci were then validated with MethyLight technology, and perfect concordance with G-CIMP calls on the array platforms versus with the MethyLight markers was observed. Consequently, similar prevalence of the phenotype was shown, providing validation of the technical performance of the platforms and of the diagnostic marker panel. Furthermore, Noushmehr and colleagues showed that G-CIMP was very tightly associated with the somatic *IDH1* mutation, and validated this in an independent subset of tumors (20).

In 2012, additional evidence for a causal role of *IDH1* in generating CIMP was presented. Using immortalized human astrocytes, Turcan and colleagues showed that the mechanistic process behind this involves the *IDH1* mutation subtly remodeling the epigenome by modulating patterns of methylation on a genome-wide scale, thereby changing transcriptional programs and altering the differentiation state (21). The authors suggest that the activity of IDH may form the basis of an "epigenomic rheostat," which links alterations in cellular metabolism to the epigenetic state (21).

Mutations in *IDH1* and *IDH2* result in a reduced enzymatic activity toward the native substrate isocitrate. Mutant IDH1 catalyzes the reduction of α-ketoglutarate to 2-hydroxyglutarate (2-HG), a potential oncometabolite (77–80) affecting gene expression via various mechanisms. This is first accomplished via competitive inhibition of α-ketoglutarate–dependent dioxygenases including Jumonji-C domain-containing histone demethylases (JHDM), thereby altering histone methylation levels. In addition, 2-HG inhibits the TET family of 5-methylcytosine (5mC) hydroxylases that convert 5mC to 5-hydroxylmethylcytosine (5hmC) via direct competition with α-ketoglutarate resulting in an accumulation of 5mC and thereby potentially altering the expression levels of large numbers of genes (49, 80). Finally, a mechanism altering hypoxia-inducible factor (HIF) expression is involved (81).

In their recent study, Turcan and colleagues showed that the expression of wild-type IDH1 caused hypomethylation at specific loci, suggesting that both the production of 2-HG and the levels of α-ketoglutarate can affect the methylome (21). Furthermore, unsupervised hierarchical clustering of methylome data showed that the hypermethylated genes included both genes that underwent *de novo* methylation as well as genes that originally possessed low levels of methylation but subsequently acquired high levels of methylation. Control astrocytes did not undergo these methylome changes. Mutant *IDH1*-induced remodeling of the methylome was reproducible and resulted in significant changes in gene expression (21).

Leukemia

For leukemia, the same story can be told. CIMP, defined by methylation of candidate genes, was reported in 2001 and 2002 (33, 36). However, the mutational and epigenetic profiling data of Figueroa and colleagues in acute myelogenous leukemia (AML) for the first time identified a causal relationship between *IDH1*, *IDH2*, and *TET2* mutations and (overlapping) hypermethylation profiles and global hypermethylation (49). Functional support for this relationship was provided *in vitro* in hematopoietic cells in which expression of mutant *IDH1* and *IDH2* leads to an increase in DNA methylation, indicating that *IDH1/2* and *TET2* mutations contribute to leukemogenesis through a shared mechanism that disrupts DNA methylation. *In vivo* evidence comes from a conditional *IDH1(R132H)* knockin mouse model, which develops increased numbers of early hematopoietic progenitors, splenomegaly, and anemia with extramedullary hematopoiesis. These alterations are accompanied by changes in DNA and histone methylation profiles (82).

Echondroma and spindle cell hemangioma

Supporting the hypothesis that *IDH1* mutation leads to DNA methylation, evidence shows that somatic mosaic mutations in *IDH1,* and to a lesser extent *IDH2*, cause enchondroma and spindle cell hemangioma in patients with Ollier disease and Maffucci syndrome (66, 83). These are rare skeletal disorders in which there is also an increased incidence of glioma (66). Using Illumina HumanMethylation27 BeadChips, Pansuriya and colleagues examined possible differences in methylation between enchondromas with and without *IDH1* mutations. Unsupervised clustering of the 2,000 most variable CpG methylation sites gave two subgroups, one of which showed an overall higher methylation at the examined CpG sites, and all but one enchondromas with an *IDH1* mutation were positive for this "CIMP" (83).

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IDH mutations in other cancer types

In addition to glioma (>70%), leukemia (AML: 15%–30%), echondroma (87%), and spindle cell hemangioma (70%), somatic *IDH1* mutations are also found in sporadic chondrosarcoma (~50%; refs. 49, 84) and at lower frequencies in anaplastic thyroid carcinoma (11%; ref. 85), (intrahepatic) cholangiocarcinomas (10%–23%; refs. 86, 87), and melanoma (10%; ref. 88), whereas in other solid tumors *IDH1* mutations are infrequent (<5%) or absent (89, 90). Interestingly, the *IDH1/2* mutations in melanoma are also accompanied by a loss of 5hmC in melanoma progression (67). Therefore, it is interesting to speculate whether or not future research to establish the cause of CIMP in other cancer types should focus on genes that are functionally similar to the *IDH* family, such as *TET2*, or on totally different genes. More specifically, it remains uncertain whether CIMP in other cancer types is also caused by inhibition of the conversion of 5mC to 5hmC and subsequent demethylation or that other factors are responsible for the accumulation of 5mC. In addition to colorectal cancer, another tumor type lacking *IDH1/2* mutations, but with a putative CIMP phenotype, is breast cancer.

Breast cancer

To date, research that has investigated CIMP in breast cancer has not been conclusive (9, 91–94), with some studies going so far as saying that CIMP does not exist in breast cancer as a truly defined phenotype (9). Recently, Fang and colleagues used unsupervised hierarchical clustering from data collected with the Infinium Human Methylation27 platform in an attempt to clarify this dispute (10). Two DNA methylation clusters in a sample of breast cancer with diverse metastatic behavior were identified. One cluster encompassed a portion of hormone receptor $(HR)^+$ tumors [defined as estrogen receptor $(ESR1)^+$/progesterone receptor $(PGR)^+$, cluster 2] and one encompassed tumors that were $ESR1^+/PGR^+$ or $ESR1^-/PGR^-$ (cluster 1). Cluster 2 tumors had a highly characteristic DNA methylation profile with high coordinate cancer-specific hypermethylation at a subset of loci, similar to the CIMP phenotype seen in colorectal cancer. They referred to this as "B-CIMP," and confirmed the composition of the phenotype through two independent clustering algorithms (10). Although intriguing, these results should be interpreted with caution. Only 39 tumors were examined in the genome-wide study, and 3 genes were chosen to validate the importance for outcome only. Furthermore, the definition for CIMP using these 3 genes could be interpreted as arbitrary, and the findings have yet to be validated in a separate cohort.

Nevertheless, this study provides interesting and considerable data for future studies. For the first time, the question of whether CIMP targeted the same genes in different human tumor types was examined by repeating the hierarchical clustering to assess colon cancer (C-CIMP) and gliomas (G-CIMP) in additional tumor samples. With this analysis, Fang and colleagues showed that there was large-scale consensus between CIMP genes from the three cancer-types. CIMP in these different malignancies seemed to target many of the same genes, suggesting a common mechanistic foundation. However, despite the observed similarities, there was not 100% overlap between the polycomb group (PcG) targets that comprise the B-, C-, and G-CIMP, which may reflect a degree of tissue or organ specificity (10). Although this supports the idea that *IDH1* mutation has been determined as the cause of G-CIMP, this is not true for other cancers. The findings must be validated in additional cohorts before firm conclusions can be made.

CIMP as a Prognostic Marker

Through their methodology, the studies of Fang and colleagues (10) and Noushmehr and colleagues (20) were able to clearly show distinct clinical characteristics of tumors characterized by B-CIMP and G-CIMP. For instance, B-CIMP tumors were associated with ESR1/PGR status, a lower risk of metastasis, and an improved clinical outcome (10). G-CIMP has been associated with improved survival, younger age at diagnosis, and histologic characteristics (20, 22). Furthermore, using the Infinium array, a recent methylome analysis in a study of patients with primary clear cell renal carcinoma showed that CIMP characterized a specific cluster of tumors associated with aggressiveness and patient outcome (32). Such findings reiterate that a major motivation for establishing whether CIMP is universal or cancer specific is because of its potential use as a prognostic marker.

Table 2 shows that CIMP is associated with both favorable and unfavorable prognosis, as well as different clinical characteristics, depending on the type of tumors. There are several possible explanations for these discrepancies. First, although CIMP has been identified in different types of cancer, it may simply not be a universal marker of good or bad prognosis. Second, as previously noted, it could be possible that for some cancers, the gene panels and cutoff thresholds used to define CIMP are not accurate for defining the "true" phenotype. It is interesting to observe that CIMP is associated with a favorable prognosis for colorectal cancer and gliomas, two cancer types for which extensive research has been conducted with respect to identifying genes that are associated with clinical and molecular features of the tumors, and in studies that included a relatively large number of cases (20, 57).

Moreover, it has been noted that the association of methylation at CIMP genes with good clinical outcome is not universally applicable to methylation at all genes. Methylation of specific candidate genes or groups of genes has been associated with poorer prognosis, and these genes may have an effect on tumor aggressiveness independent of CIMP (10).

Conclusions and Future Perspectives

Much like what has been observed in the field of colorectal cancer research (7), the study of CIMP in other tumor types has been quite heterogeneous in terms of how the phenotype has been defined. Recent studies considering genome-wide characterization of the methylome in gliomas and leukemia have shown that CIMP is likely more than just a generic name to be used to describe aberrant methylation.

Although there is some overlap with respect to genes targeted by CIMP in colon cancer, breast cancer, and gliomas, and although *IDH1* and genes that affect the same (metabolic) pathway, such as *IDH2* and *TET2*, have been shown to be

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Table 2. CIMP and clinicopathologic features of different cancers

Cancer type	Significant clinical associations	Prognosis
Adrenocortical carcinomas (39)		–
Bladder cancer (8)		–
Breast cancer (10)	Subset of hormone positive tumors ($ESR1^+/PGR^+$)	+
Colorectal cancer (56)	Female	+
	Older age	
	Proximal location	
	MSI	
	BRAF mutation	
Duodenal adenocarcinomas (38)		
Endometrial cancer (12, 13)	Early stage	–
	COX-2 hypermethylation	
Gastric cancer (14–19)	MSI	±
	Lymph node metastasis	
Gliomas (20)	Younger age at diagnosis	+
	IDH1 mutation	
Hepatocellular carcinoma (23–26)	Serum α-fetoprotein (AFP)	–
	Metastasis	
	TNM staging	
	CIMP in serum	
Leukemia (adult acute lymphocytic; ref. 33)	Younger age	
Leukemia (acute myeloid; ref. 36)	Younger age	
Leukemia (T-cell acute lymphoblastic; ref. 35)		+
Leukemia (childhood acute lymphoblastic; ref. 34)		–
Lung cancer (27, 28)		–
Melanoma (37)	Advanced stage	–
Neuroblastoma (40, 41)		–
Prostate cancer (31)	High preoperative serum (PSA) levels	–
	Advanced stage	
Renal cell carcinoma (32)	Tumor aggressiveness	–

causally involved in the generation of CIMP in gliomas and leukemia, cancer-specific differences still exist and the cause of CIMP in the majority of cancer types remains to be identified. The causal relationship between somatic mutations in genes such as *IDH1*, *IDH2*, and *TET2* and altered genome-wide DNA methylation profiles generated by next-generation sequencing techniques is a promising clue on the cause of CIMP. The fact that these mutations impair histone demethylation and induce repressive histone methylation marks thereby blocking cell differentiation (95) provide clues on the complex relations between specific genetic alterations, CIMP, and clinical characteristics such as histologic features and prognosis.

In addition, analyzing the relationship between somatic mutations in chromatin remodeling genes and CIMP could yield interesting insights. For example, *AT-rich interactive domain-containing protein 1a* (*ARID1a*), a member of the switch/sucrose nonfermentable (SWI-SNF) complex, has been reported to be mutated and inactivated in a subset of gastrointestinal cancers, the majority of which also exhibit another characteristic of C-CIMP, namely MSI (96–98).

To unify the field and to establish a standard definition for CIMP, we present the following recommendations:

1. CIMP is not a single phenotype in all types of cancer. A simple variation from the standard nomenclature of "CIMP" to make this distinction, such as "C-CIMP" for colorectal cancer CIMP, "G-CIMP" for glioma CIMP, "L-CIMP" for leukemia CIMP, and "B-CIMP" for breast cancer CIMP should be adopted for clarity.
2. Multiple reports suggest a third category of CIMP in colorectal cancer by dividing CIMP into CIMP-high and CIMP-low. Although CIMP-low has repeatedly been associated with KRAS mutations, this group has many clinical and pathologic features in common with non-CIMP, and thus without evidence that this is a distinct phenotype and without consensus on how to define CIMP-low, the use of CIMP-low should be discouraged.
3. A consensus meeting should be organized to:
   a) Obtain recommended guidelines on the optimal CIMP marker panel for each tumor type. This includes the number of markers in the panel, the specific loci (genes) included, and the defined region examined for methylation in each gene.
   b) Obtain recommended guidelines on the method to measure CIMP. If quantitative methods are needed for CIMP classification, defined cutoffs must be established for each marker for subsequent validation.

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4. Once CIMP markers have been identified, they should be validated in large, independent, well-characterized patient series with clinical follow-up data (molecular pathologic epidemiology approach; refs. 99, 100).
5. A research effort for identifying the biologic cause of CIMP among tumor types should be implemented once standard criteria for CIMP are established and validated. Focus should be on establishing causal relationships to find the driver(s) of CIMP.
6. Dissemination of the recommended guidelines to the field, as was done for Bethesda MSI markers (101), is crucial in standardizing research in the field of CIMP.

Hopefully, these recommendations will help to establish the true causes, manifestation, and proper definitions of CIMP.

Disclosure of Potential Conflicts of Interest

W. van Criekinge is employed as CSO in MDX Health. J.G. Herman has a commercial research grant from MDX Health and is a consultant/advisory board member of the same. M. van Engeland has a commercial research grant from MDx Health. No potential conflicts of interest were disclosed by the other authors.

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