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Review

Biological significance of the CpG island methylator phenotype

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ABSTRACT

Cancers exhibiting the CpG island methylator phenotype (CIMP) are found among a wide variety of human malignancies and represent a subclass of tumors showing concurrent hypermethylation of multiple CpG islands. These CIMP-positive tumors often exhibit characteristic molecular and clinicopathological features, suggesting CIMP represents a distinct carcinogenic pathway. However, marker genes to define CIMP have been largely inconsistent among studies, which has caused results to vary. Nonetheless, recent advances in genome-wide methylation analysis have enabled the existence of CIMP to be confirmed, and large-scale cancer genome analyses have begun to unravel the previously unknown molecular basis of CIMP tumors. CIMP is strongly associated with clinical outcome, suggesting it may be a predictive biomarker.

Contents

1. Introduction

Fifteen years have passed since the first report of the CpG island methylator phenotype (CIMP) was published [1]. It is now known that hypermethylation of CpG islands (CGIs) at gene promoters plays a key role in the silencing of numerous cancer-related genes affecting a variety of vital cellular processes, and that CIMP-tumors represents a subtype of cancers that exhibit concurrent hypermethylation of multiple CGIs. CIMP was first documented in colorectal cancer (CRC), and because of its characteristic molecular and clinicopathological features, CIMP was thought to represent a distinct pathway of colorectal carcinogenesis [1,2]. Subsequently, methylation of multiple CGIs was also reported in other cancers, including gastric [3,4], esophageal [5], hepatic [6,7], pancreatic [8], lung [9], ovarian [10], renal [11], duodenal [12] and oral cancers [13], as well as malignant melanoma [14], neuroblastoma [15,16] and hematological malignancies [17–19]. CIMP-positive groups thus exist in a wide variety of human malignancies (Table 1).

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Table 1
CIMP in human malignancies.

Tumor type	Subclass	Frequency (%)	Biological significance	Prognosis	Reference
Colorectal cancer	CIMP-high	11–23	MSI, BRAF mutation	Good	[43–46,53]
	CIMP-low	23–40	KRAS mutation	Poor	
Oral squamous cell carcinoma		19–56		Poor	[13,25]
Esophageal squamous cell carcinoma		54		Poor	[5]
Gastric cancer	CIMP-high	15–41	EBV infection	Controversial	[3,4,94–96,101]
	CIMP-low	21–78			
Duodenal adenocarcinoma		27		Poor	[12]
Hepatocellular carcinoma		62–80	High telomerase activity and serum AFP	Poor	[6,7]
Breast cancer		44		Good	[20]
Lung adenocarcinoma	CIMP-high	8		Poor	[21]
	CIMP-low	31			
Glioblastoma		9	IDH1 mutation	Good	[27]
Neuroblastoma		33–48	N-myc amplification	Poor	[15,16]
Paraganglioma		12	SDHx mutation	Poor	[28]
Clear cell renal carcinoma		13		Poor	[26]
Bladder carcinoma		34			[24]
T-cell ALL		76		Poor	[18]
T-cell ALL (pediatric)		49		Good	[108]
pre-B-cell ALL (ETV6/RUNX1-positive)		67		Poor	[107]
Myelodysplastic syndrome				Poor	[19]
Adult T-cell leukemia/lymphoma				Poor	[109]

ALL, acute lymphoblastic leukemia.

Despite its apparently wide distribution of CIMP, it has been difficult to define the methylator phenotype due to the ambiguous borderline between CIMP-positive and CIMP-negative groups. This ambiguity stems mainly from an absence of consistent criteria by which to define CIMP; in particular, there is substantial inconsistency among studies with respect the marker genes and analytical methods used to define CIMP. To address these issues, numerous investigators have put much effort into identifying marker genes that accurately characterize CIMP positive-tumors in CRC. Moreover, in recent years technological advances have enabled genome-wide DNA methylation analysis, and unsupervised clustering of the methylome data has clearly delineated CIMP-positive groups in breast [20], lung [21], colorectal [22], endometrial [23], bladder [24] oral [25] and renal cancer [26], as well as in glioma [27], paraganglioma [28] and ependymoma [29] (Table 1).

The identification of CIMP could potentially lead to better understanding of the molecular basis of tumorigenesis as well as improved treatment of cancer patients. For instance, the recent discovery of *IDH1/2* and *TET2* gene mutations in CIMP-positive tumors suggested their causal relationship [27,30]. In addition, several studies have reported an association between CIMP and clinical outcome, suggesting that CIMP could be a predictive marker for patient survival or chemosensitivity (Table 1). In this review, we will summarize our understanding of CIMP in various malignancies and highlight its biological and clinical significance.

2. Discovery of CIMP in CRC

CIMP was first identified through a genome-wide screen of methylated CGIs in CRC cells. Using methylated CpG island amplification (MCA) coupled with representational difference analysis (RDA), Toyota et al. compared the CGI methylation statuses of the CaCO2 CRC cell line and normal colonic mucosa samples. They identified 30 CGIs hypermethylated in CRC, which they termed MINT (methylated in tumors) clones [1,31]. The majority of the CGIs (19/30) were methylated in both tumors and normal colonic tissue in an age-dependent manner (type A methylation), while a small number (7/30) were methylated in a cancer-specific manner (type C methylation). By analyzing MINT loci with type C methylation in primary tumors, they identified a subset of CRCs that showed frequent hypermethylation at multiple loci. These CIMP-positive CRCs were then further divided into two groups based on *MLH1* methylation and microsatellite instability (MSI) status, and a tight association between *MLH1* methylation and MSI was observed (Table 2).

Molecular signatures and clinicopathological features of CIMP have been investigated by a number of groups. Taken together, their findings show that CIMP-positive CRCs are strongly associated with a proximal colon location, older age, female sex, higher tumor grade, poorly differentiated and mucinous histology, *KRAS*/*BRAF* mutation and wild-type *TP53* [1,2,32–34]. CRCs are known to exhibit one of two genetic instabilities, MSI or chromosomal instability (CIN). And whereas there is a significant correlation between CIMP and MSI, large chromosomal aberrations or loss of heterozygosity (LOH) are less frequently observed in CIMP-positive CRCs [35–37].

The CIMP hypothesis has been questioned by several groups who found that CRCs could not be clearly categorized into CIMP-positive and CIMP-negative groups [38,39]. Yamashita et al. reported that the numbers of methylated CGIs vary discontinuously from the high-methylation group to the low-methylation group, which is in sharp contrast to the bimodal distribution of microsatellite mutations found in MSI and microsatellite stable (MSS) cancers [38]. They also showed that many of the hypermethylation events are age-dependent and concluded that the mutator phenotype is dominant with respect to the methylator phenotype. Such complexity and controversy stems mainly from differences in the marker genes and analytical methods used to define CIMP tumors [40]. Many of the earlier studies used highly sensitive and qualitative methods to analyze arbitrarily selected tumor-related genes, which may lead to overestimation or erroneous categorization of CIMP tumors. By contrast, CIMP was originally defined through quantitative methylation analysis (using COBRA assay), and subsequent studies confirmed that quantitative analysis is likely the key to accurate characterization of CIMP [1,41,42].

To address the controversy, a substantial effort has been made to identify the best markers with which to define CIMP. For instance, Weisenberger et al. used a quantitative MethyLight assay to analyze the methylation status of 195 CGIs in a cohort of 295 primary CRC tumors [42]. They showed that unsupervised clustering analysis using CGIs with cancer-specific methylation could confirm the existence of CIMP-positive tumors. As compared to

Table 2
Representative studies of CIMP in CRC.

Author	Subclass	Marker genes	Cutoff	Frequency	Molecular and clinical features	Reference
Toyota et al.		MINT1, MINT2, MINT12, MINT17, MINT25, MINT27, MINT31	⩾3/7	28/49 (57%)	p16, THBS1, MLH1 methylation, MSI, KRAS mutation, wild type p53, proximal colon	[1,2]
Hawkins et al.		MINT1, MINT2, MINT12, MINT31, CDKN2A (p16)	⩾2/5	76/402 (19%)	MSI, KRAS mutation, wild type p53, proximal colon, female, older age, high tumor grade, mucinous type	[33]
Samowitz et al.		MINT1, MINT2, MINT31, CDKN2A (p16), MLH1	⩾2/5	256/864 (30%)	MSI, KRAS, BRAF mutation, wild type p53, proximal colon, older age, increased stage	[34]
Goel et al.		MINT1, MINT2, MINT31, CDKN2A (p16), p14, MLH1	⩾3/6	39/126 (31%)	BRAF mutation, MSI or MSI-/LOH-	[35]
Ogino et al.		CACNA1G, CDKN2A (p16), CRABP1, MLH1, NEUROG1	⩾4/5	78/460 (17%)	MSI, BRAF mutation	[41]
Weisenberger et al.		CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1	⩾3/5	33/187 (18%)	MLH1 methylation, MSI, BRAF mutation, proximal colon	[42]
Ogino et al.	CIMP-high	CACNA1G, CDKN2A (p16), CRABP1, MLH1, NEUROG1	⩾4/5	130/840 (15%)	BRAF mutation, wild type p53, female	[43]
	CIMP-low	Same as CIMP-high	1/5 to 3/5	279/840 (33%)	KRAS mutation, male	
Ogino et al.	CIMP-high	CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1, CDKN2A (p16), CRABP1, MLH1	⩾6/8	136/920 (15%)		[53]
	CIMP-low	Same as CIMP-high	1/8 to 5/8	353/920 (38%)		
Shen et al.	CIMP1	MLH1, TIMP3, MINT1, RIZ1, BRAF mutation		22/97 (23%)	MSI, BRAF mutation, wild type p53	[44]
	CIMP2	KRAS mutation		37/97 (38%)	KRAS mutation, wild type p53	
Yagi et al.	HME	CACNA1G, LOX, SLC30A10	⩾2/3	17/149 (11%)	MSI, BRAF mutation	[45]
	IME	CACNA1G, LOX, SLC30A10 and ELMO1, FBN2, THBD, HAND1, SLC30A10	0/3 or 1/3 and ⩾3/5	60/149 (40%)	KRAS mutation	
Hinoue et al.	CIMP-high	FAM78A, FSTL1, KCNC1, MYOCD, SLC6A4 and B3GAT2, FOXL2, KCNK13, RAB31, SLIT1	⩾3/5 and ⩾3/5	28/125 (22%)	MLH1 methylation, BRAF mutation	[46]
	CIMP-low	FAM78A, FSTL1, KCNC1, MYOCD, SLC6A4 and B3GAT2, FOXL2, KCNK13, RAB31, SLIT1	0/5 to 2/5 and ⩾3/5	29/125 (23%)	KRAS mutation	

HME, high-methylation epigenotype; IME, intermediate-methylation epigenotype.

the classic CIMP markers (*MINT1*, *MINT2*, *MINT31*, *CDKN2A* (p16) and *MLH1*), CIMP-positive tumors defined using the new markers identified in that study (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3* and *SOCS1*) are more tightly associated with MSI and *BRAF* mutation, suggesting that mismatch repair deficiency is a consequence of CIMP-associated silencing of *MLH1*.

3. Subclasses of CIMP in CRC

Although the tight association between CIMP and MSI has been repeatedly documented, studies have also revealed that there are distinct subclasses of CIMP-positive CRCs (Table 2). Ogino et al. analyzed a panel of 5 CIMP-specific genes (*CACNA1G*, *CDKN2A*, *CRABP1*, *MLH1* and *NEUROG1*) in 840 population-based CRC patients [43]. They found that CRCs with intermediate methylation, termed CIMP-low (defined as 1/5 to 3/5 methylated genes), are strongly associated with male sex and *KRAS* mutation, which is not the case with CIMP-high tumors (defined as 4/5 or 5/5 methylated genes). Shen et al. carried out unsupervised hierarchical clustering of 97 primary CRCs using 27 methylation marker genes, and showed that CRCs could be classified into 3 subclasses: CIMP1, which is strongly associated with MSI and *BRAF* mutation; CIMP2, which is characterized by *KRAS* mutation; and CIMP-negative, which is associated with frequent *TP53* mutation [44]. Similarly, Yagi et al. used 44 newly identified marker genes and reported that CRCs could be classified into high-, intermediate- and low-methylation epigenotypes (HME, IME, LME) [45]. In recent years, more comprehensive analysis using DNA methylation arrays (Illumina Infinium DNA methylation assay) confirmed 2 distinct CIMP subclasses comparable to CIMP-high and CIMP-low [22,46].

CIMP-high (or CIMP1) CRCs show the well-known characteristics of CIMP-positive tumors: proximal location, older age, female sex, frequent *BRAF* mutation, *MLH1* methylation and MSI, and rarely *KRAS* and *TP53* mutation [44–46]. The reason for the frequent *MLH1* methylation in CIMP-high is not yet clear. A recent study reported that a polymorphism in the promoter of *MLH1* (c.-93G>A SNP [rs1800734]) is associated with an increased risk of MSI-high CRC [47]. Two other groups then analyzed the association between this genotype and *MLH1* methylation in CRC, but obtained differing results [48,49]. The mechanism of the tight association between CIMP-high and *BRAF* mutation also remains unclear, and ectopic expression of oncogenic BRAF ($BRAF^{V600E}$) in CIMP-negative CRC cells did not specifically induce CGI methylation [50]. Recently, IGFBP7 was found as a downstream effector of oncogenic BRAF that induces cellular senescence and apoptosis in melanocytes, and that inactivation of *IGFBP7* through promoter CGI methylation is a critical step in the pathogenesis of melanoma [51]. Methylation of *IGFBP7* also frequently occurs among CIMP-high CRCs, suggesting that loss of IGFBP7 may be necessary to escape from oncogenic BRAF-induced senescence during colorectal carcinogenesis [50,52].

CIMP-low (or CIMP2) tumors are strongly associated with *KRAS* mutation, whereas MSI, *BRAF* and *TP53* mutation is less frequent [43–46]. Preferential location in the proximal colon is also observed with CIMP-low tumors [44,46], but they are slightly more common in men than in women [43,46]. Other molecular and clinical characteristics of CIMP-low tumors sometimes differ among studies, most likely due to differences in the criteria used to define this subclass. Ogino et al. evaluated 8 CIMP marker genes (Weisenberger's new CIMP markers plus *CDKN2A*, *CRABP1* and *MLH1*) and found that differences between CIMP-low (1/8 to 5/8 methylated genes) and CIMP-negative are not clear-cut, indicating that these markers are more specific to CIMP-high [53]. In addition, Shen et al. concluded that the single best marker for predicting CIMP2 (CIMP-low) is *KRAS* mutation [44].

To identify CIMP subclasses more specifically, Hinoue et al. proposed using two panels of markers consisting of commonly

methylated genes in CIMP-high and -low tumors and additional CIMP-high-specific genes [46]. Yagi et al. also developed a similar two-panel method to distinguish the three epigenotypes [45]. However, a very recent study suggested that IME/LME tumors may not be equivalent to CIMP-low/CIMP-negative tumors [54]. Although multiple lines of evidence support the existence of CIMP subcategories, one must keep in mind that differences in marker genes can result in significant discrepancies among studies.

4. Possible mechanisms to induce CIMP in CRC

The mechanism by which aberrant DNA methylation is induced in CRC remains largely unknown, though several factors that may be causally involved have been reported. For instance, one recent study reported overexpression of DNA methyltransferase-3B (DNMT3B) in CIMP-high tumors [55]. DNMT3B expression is also reportedly increased during colorectal neoplastic progression, and its expression correlates positively with the levels of methylation of the CIMP-associated genes (*NEUROG1*, *CACNA1G* and *CDKN2A*) as well as *SFRP2*, suggesting a causal relationship [56].

The association of lifestyle and genetic factors with CIMP CRCs has also been investigated in several epidemiological studies. Cigarette smoking is reportedly associated with CIMP-high and *BRAF* mutant CRCs [57,58], and dietary factors are also believed to play important roles in tumorigenesis. One early study examined the possible effects of low-folate and high alcohol intake on promoter methylation in CRC [59]. Folate plays a crucial role in DNA metabolism and synthesis, and it is required to maintain an adequate cellular pool of the methyl donor S-adenosylmethionine (SAM). Several genetic polymorphisms in folate metabolizing enzymes (*MTHFR*, *MTR* and *MTRR*) are reportedly associated with CIMP status in CRC [60,61], and certain *MTHFR* polymorphisms are positively associated with CIMP when they are present in conjunction with a high-risk dietary pattern (low folate and methionine intake and high alcohol use) [62]. On the other hand, there is little evidence supporting an association between dietary folate, vitamins B_6 and B_{12}, methionine or alcohol intake and CIMP-high tumors [63,64]. Further study will be necessary to clarify the role of dietary methyl donor intake and aberrant DNA methylation in cancer.

Although no genetic mutation of DNMTs or histone modifying enzymes has been reported in CIMP-positive CRCs, a recent whole-exome sequencing analysis in CIMP1 (CIMP-high) tumors identified recurrent mutation of *CHD7* and *CHD8*, which encode members of the chromatin remodeling family [65]. Mutation of *CHD7* and *CHD8* occurs more frequently in CIMP-high tumors than in other tumors, and many of the mutations are predicted to truncate or damage the protein. Interestingly, genes frequently methylated in CIMP-positive CRCs are enriched among CHD7-regulated genes in neural stem cells, indicating a possible role for this aberration in the pathogenesis of CIMP-high CRCs.

5. Clinical significance of CIMP in CRC

DNA methylation could be a useful biomarker for cancer risk assessment, detection and outcome prediction. The impact of CIMP on the clinical outcome of CRC patients has been analyzed fairly extensively, but the results have been inconsistent. Multiple lines of evidence suggest that CIMP-high with MSI is a marker of a favorable prognosis in CRC patients, while CIMP in MSS tumors is an indicator of poor survival. Using the classic CIMP markers (*MINT1*, *MINT2*, *MINT12*, *MINT31*, *CDKN2A* and *MLH1*), Ward et al. analyzed more than 600 CRC patients and found that no single marker was independently associated with prognosis [66]. However, when they divided the CRC patients into MSI and MSS groups, methylation of multiple (>3/5) genes was significantly associated with an adverse prognosis in patients with MSS tumors. Later studies similarly showed that outcomes were worse among patients with tumors showing CIMP-positivity and MSS [67,68]. An association between CIMP and shortened survival was also observed in advanced CRC patients, among whom the contribution of MSI is relatively limited [69]. When CIMP tumors were divided into 2 subclasses, CIMP-high status in MSS tumors was again strongly associated with shorter survival, while CIMP-low may be an indicator of poor outcome regardless of MSI [67,70]. Similarly, the intermediate-methylation epigenotype with KRAS mutation is also reportedly associated with poor outcome [45].

Simons et al. Recently classified 509 CRC samples from the Netherlands Cohort Study based on their CIMP, MSI and CIN status: MSI, CIMP-only, CIMP plus CIN, CIN only and triple-negative [71]. They found that the CIMP-only, CIMP plus CIN and triple-negative groups were significantly associated with higher incidence of CRC-related death than CIN-only tumors. The value of DNA methylation as a prognostic marker may be influenced by other clinical factors or genetic backgrounds. In the proximal CRCs, CIMP1 (CIMP-high) is correlated with a higher recurrence rate than CIMP2 (CIMP-low) or CIMP-negative tumors, but a similar correlation was not found with distal CRC [72]. Another study reported that CIMP-high contributed to a poor prognosis only in rectal cancers in Asian populations [73].

The above results indicate that CIMP is predictive of a poorer prognosis, though the influence may be lost in MSI-positive tumors. However, other lines of evidence suggest the adverse effects could be attributable to *BRAF* mutation. By analyzing a large cohort of CRCs, Ogino et al. showed that CIMP-high is an independent predictor of low colon cancer-specific mortality, regardless of MSI of *BRAF* status, while *BRAF* mutation is strongly associated with high cancer-specific mortality [74]. A poorer prognosis with *BRAF*-mutated MSS tumors was also reported in two other independent studies [75,76]. In addition, Dahlin et al. analyzed two independent sets of large CRC cohorts, the Northern Sweden Health and Disease study (NSHDS) and the Colorectal Cancer Umeå Study (CRUMS), and found an association between *BRAF* mutation and a poor prognosis in the NSHDS but not the CRUMS [70].

Cumulative evidence indicates that MSI-high CRCs have a better prognosis, but no benefit is obtained from 5-fluorouracil (5-FU)-based adjuvant chemotherapy with these patients [77–79]. A few studies have assessed the benefit of chemotherapy in CIMP-positive CRCs, but the results have been inconsistent. One earlier report showed that CIMP is an independent predictor of survival benefit from 5-FU in stage III CRCs, though the investigators used only 3 genes to define CIMP, which may have caused inaccurate classification of the tumors [80]. More recently, two groups from Western and Asian countries analyzed the survival benefit from 5-FU adjuvant chemotherapy in patients with CIMP-positive stage II–III CRC and obtained opposite results [81,82]. Although those investigators employed similar panels of marker genes to define CIMP, one found no benefit from 5-FU [79], while the other observed longer recurrence-free survival in the 5-FU treated group [80]. There are several possible explanations for this discrepancy, including differences in the methods and ethnicity, but further study will be needed to clarify the responsiveness of CIMP tumors 5-FU chemotherapy.

6. CIMP in colorectal premalignant lesions

It is well documented that aberrant DNA methylation occurs early during tumorigenesis, and multiple studies have shown the presence of CIMP in colorectal adenomas and hyperplastic polyps

(HPs) [1,83–85]. For a long time, HPs were considered to be colorectal lesions with little neoplastic potential, and therefore of little pathogenic consequence. However, the recent proposal of the "serrated pathway," which can progress to CIMP-positive CRCs, has challenged this view [86]. Serrated lesions include goblet cell HPs, microvesicular HPs, sessile serrated adenoma/polyps (SSA/Ps) and traditional serrated adenomas (TSAs). SSA/Ps are strongly associated with a proximal colon location, *BRAF* mutation and CIMP-high, and they are considered to be precursor lesions for CIMP-high/MSI CRCs [86,87].

When evaluating their location, most studies classify CRCs as rectal, distal colonic or proximal colonic, and it is not yet clear whether the molecular characteristics of CRCs, especially those of CIMP-high tumors, differ between proximal and distal colonic tumors. One recent study showed that the incidence of CIMP-high, MSI-high, *BRAF* mutant cancers gradually increases along the bowel, from the rectum to the ascending colon [88]. Interestingly, a recent analysis of serrated lesions also revealed a gradual increase in the incidence of CIMP-high tumors from the rectum to the cecum, but *BRAF* mutation was observed in lesions throughout the rectum and colon, indicating a site-dependent difference in the susceptibility to CIMP and *BRAF* mutation in premalignant lesions [89].

Detection and removal of SSA/Ps during colonoscopies would contribute to reducing CRC mortality, but it is often difficult to discriminate SSA/Ps from HPs through colonoscopic observation, as neither shows the surface microstructure (pit pattern) specific to malignant lesions. A recent population study reported that use of colonoscopy and sigmoidoscopy could reduce both the incidence of CRC and its mortality, while cancers diagnosed after 5 years are more likely to have CIMP and MSI, which may reflect the difficulty of detecting SSA/Ps during colonoscopy [90]. We recently found an SSA/P-specific pit pattern, Type II-O, which could improve colonoscopic detection of SSA/Ps [91]. We also carried out an integrative genome and epigenome analysis, and found that both *BRAF*/*KRAS* mutation and CIMP occur in premalignant lesions, while *MLH1* methylation (and MSI) and copy number aberrations (CIN) are acquired during the progression from adenoma to carcinoma [92]. We would anticipate that further advances in our knowledge of premalignant lesions would lead to better prevention and earlier detection of CRCs.

7. CIMP in other malignancies

7.1. CIMP in gastric cancer

Aberrant DNA methylation is reportedly involved in the development of gastric cancer (GC). *Helicobacter pylori* (*H. pylori*) infection is associated with an increased risk of GC, and CGI hypermethylation has been found in *H. pylori*-induced gastritis, suggesting methylation is an early event during gastric tumorigenesis [93]. CIMP-positive tumors have also been reported in GC. The earliest study used classic CIMP markers and found that approximately 40% of primary GCs are CIMP-positive [3]. A link between *MLH1* methylation, MSI and CIMP was also observed in GCs, but that occurs only in a fraction of CIMP-positive (even among CIMP-high) tumors [3,4,94].

Interestingly, Epstein–Barr virus (EBV)-associated GCs are found exclusively in the CIMP-high group [94,95], and introduction of recombinant EBV induces DNA methylation in EBV/CIMP-negative GC cells [96]. EBV belongs to the Herpesviridae family. It causes infectious mononucleosis upon initial infection and is also involved in several malignancies, including Burkitt lymphoma, nasopharyngeal carcinoma and GC [97]. In general, genes methylated in cancer cells are targets of the polycomb repressive complex (PRC) in embryonic stem cells [98], but this is not the case for genes specifically methylated in EBV-associated GCs, indicating that a different mechanism is involved in EBV-induced DNA methylation [96].

The association between CIMP and clinical outcome of GC patients has been investigated by several groups, but results are inconsistent; some reported better survival among patients with CIMP-positive GC [4,94,99], while others found a significantly worse prognosis in the CIMP-high patients [100,101]. In addition, a recent meta-analysis concluded that CIMP cannot be a prognostic marker of GC [102]. However, considering the large differences in the marker genes used in these studies and the relatively limited numbers of patients analyzed by the respective groups, we suggest further study will be necessary to clarify the clinical significance of CIMP in GC.

7.2. CIMP in brain tumors

The importance of aberrant DNA methylation in the pathogenesis and clinical treatment of brain tumors has been investigated by a number of groups. It is well documented, for instance, that *MGMT* promoter methylation can serve as a predictive biomarker for the responsiveness of glioma to alkylating chemotherapy [103]. In addition, a recent analysis of the glioblastoma (GBM) genome data obtained by The Cancer Genome Atlas (TCGA) project revealed that a subset of GBMs exhibit CIMP (called glioma-CIMP or G-CIMP) [27]. G-CIMP is associated with a younger age at diagnosis and better outcome and, more strikingly, it is tightly associated with *IDH1* mutation. Expression of mutant IDH1 protein results in the production of an oncometabolite, 2-hydroxyglutarate (2-HG), and induction of mutant IDH1 in astrocytes induces extensive DNA hypermethylation [104]. Mutant IDH1 inhibits α-ketoglutarate (α-KG)-dependent dioxygenases, including histone demethylases and the TET family of 5-methylcytosine hydroxylases [105]. Because TET-mediated production of 5-hydroxymethylcytosine (5-hmC) is a primary mode of active DNA demethylation, inhibition of this activity may be a mechanism underlying the aberrant DNA methylation seen in *IDH1*-mutant glioma [104].

Ependymomas are common childhood brain tumors that occur throughout the nervous system, but are most common in the hindbrain. Although treatment protocols for childhood malignancies have improved over time, ependymomas are resistant to cytotoxic chemotherapies, and there are few treatment options for recurrent ependymomas. A recent genome and epigenome analysis of hindbrain ependymoma revealed an extremely low mutation rate and a strong association between CIMP and poor prognosis [29]. CIMP-positive ependymoma cells respond to epigenetic drugs, including a DNA demethylating agent and an EZH2 inhibitor, suggesting epigenetic modifiers are a potential therapeutic approach to treating ependymomas.

7.3. CIMP in hematologic malignancies

Concurrent hypermethylation of multiple CGIs has been reported in various hematological malignancies, including acute myeloid leukemia (AML) [17], acute lymphoblastic leukemia (ALL) [18,106–108], myelodysplastic syndromes (MDS) [19] and adult T-cell leukemia/lymphoma (ATLL) [109], which suggests the presence of CIMP. CIMP is associated with shorter survival in T-cell ALL (T-ALL), childhood B-cell precursor ALL, MDS and ATLL, though another study reported the opposite results for childhood T-ALL [108]. Most of these studies defined CIMP using selected tumor suppressor or tumor associated genes, and a lack of consistency in the definition of CIMP may be a cause for the conflicting results.

Recently, recurrent mutations in *IDH1*, *IDH2* and *TET2* were found in myeloid malignancies [30,110,111]. Mutations in *IDH1/2* and *TET2* are mutually exclusive, and disruption of *TET2* by mutant IDH or *TET2* loss-of-function mutation correlates with global DNA hypermethylation [30]. These results suggest that mechanisms similar to those in G-CIMP tumors may be involved in the pathogenesis of AML with the methylator phenotype.

8. Concluding remarks

Until today, studies have shown a strong association between CIMP and clinical outcome or chemosensitivity, which suggests CIMP may be a useful biomarker for some cancer patients. However, the criteria used to define CIMP remains inconsistent for most tumor types. Even among CRCs, where CIMP has been most extensively analyzed, accurate definition of CIMP-low tumors is not easy, and the relationship between CIMP and clinical outcome remains to be clarified. Additional large-scale studies with stringent criteria are strongly needed to achieve a full understanding of the biological and clinical importance of CIMP.

Thanks to recent technological advances, the presence of CIMP is being confirmed or newly identified in wide variety of malignancies. In particular, large-scale cancer genome analyses revealing CIMP-specific mutations have led to identification of previously unknown mechanisms underlying global changes in DNA methylation. Although not mentioned in this review, one recent study showed that mutation of *SDH*, which encodes tricarboxylic acid cycle enzymes, can also induce the methylator phenotype in paraganglioma by inhibiting histone and DNA demethylases [28]. It is not yet clear whether similar mechanisms are involved in CIMP-positive tumors in other organs, but future studies will unravel the molecular and etiological factors leading to CIMP induction.

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