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*Review*

# DNA Methylation: From Cancer Biology to Clinical Perspectives

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**Abstract**

DNA methylation plays an important role in the silence of tissue-specific genes to prevent them from being expressed in the wrong tissue. Aberrant DNA methylation (genome-wide hypomethylation and site-specific hypermethylation) are observed in many types of cancer. DNA methylation patterns are established and maintained through the combined actions of methyltransferase and demethylase, such as DNA methyltransferase (DNMT)-1, DNMT-3, and ten-eleven translocation (TET) family enzymes. It is well known that the process of tumor evolution is complicated with different hallmarks. Early findings put forward the model that focal hypermethylation of tumor suppressor genes (*TSG*) could straightly trigger transcriptional silencing and malignant transformation, whereas varying levels of DNA methylation also occur at other sites and can differently regulate gene expression and biological processes. The interplay of tumor and immune cells in the tumor microenvironment is complex. Understanding the role of DNA methylation in cancer immunity is critical to better navigate epigenetic agents. Furthermore, a greater understanding of the interaction of DNA methylation with tumor metabolic reprogramming would create a bright avenue for pharmacologic managements of malignancies. In this review, we will describe the molecular mechanisms of DNA methylation abnormalities in cancer biology, introduce the roles of DNA methylation patterns on cancer-immunity cycle and metabolic reprogramming, summarize modulators that are used in targeting DNA remodeling, and highlight the importance of combining epigenome-targeting drugs with other cancer therapies.

**Keywords:** DNA methylation; metabolism reprogramming; cancer-immunity; novel anti-cancer strategy

## 1. Introduction

Covalent epigenetic modifications made on DNA and histone cooperatively regulate chromatin structure and gene expression [1]. DNA methylation is the most well-characterized epigenetic mechanism. It plays an important role in the silence of tissue-specific genes to prevent them from being expressed in the wrong tissue. The addition and removal of methyl groups to DNA are catalyzed by specific chromatin-modifying proteins (CMP) known as 'writers' and 'erasers', which is a reversible process and is dynamically regulated [2]. DNA methylation is the covalent addition of a methyl-group (-CH3) to the cytosine (C) base that is subsequently converted to 5-methycytosine (5mC) [3,4], which is mainly catalyzed by the DNA methyltransferase (DNMT) family, including DNMT1, DNMT3A, and DNMT3B. S-adenosylmethionine (SAM) is the methyl donor provided by the methionine cycle. Conversely, the ten-eleven translocation (TET) family has been proposed to mediate DNA demethylation in an indirect manner through the oxidization of 5-methylcytosine [5].

Genetic alterations of CMPs, such as DNMT3A and TET2, are frequently observed in human cancers [6], leading to aberrant DNA methylation patterns (genome-wide hypomethylation and site-specific hypermethylation). Theoretically, focal hypermethylation of tumor suppressor genes (*TSG*) could trigger transcriptional silencing and malignant transformation. Varying levels of DNA methylation can also occur at other sites and differently regulate gene expression and biological processes [7–10]. DNA methylation supports dynamic gene expression, affecting the highly orchestrated trafficking and activation of tumor infiltrating lymphocytes [11,12]. Therefore, it is capable of modulating anti-tumor immune responses. It is known that DNA methylation is essential for T cell fate and function. A thorough understanding of the role of DNA methylation in cancer immunity is critical to better navigate epigenetic agents. Further, increasing evidence indicates that DNA methylation affects the alteration of metabolic enzymes, which is directly linked to metabolism reprogramming in oncogenesis [13].

Given the extensive alterations and functions in the DNA methylomes, DNA methylation inhibitors form the standard of care for hematological malignancies and the fundamentals of clinical trials in solid tumors [14]. Therapies that combine DNA methylation inhibitors with standard chemotherapy, metabolism modulators, and immunotherapy, will bring novel therapies to clinical decision making.

In this review, we outline the molecular mechanism of DNA methylation abnormalities in cancer biology,

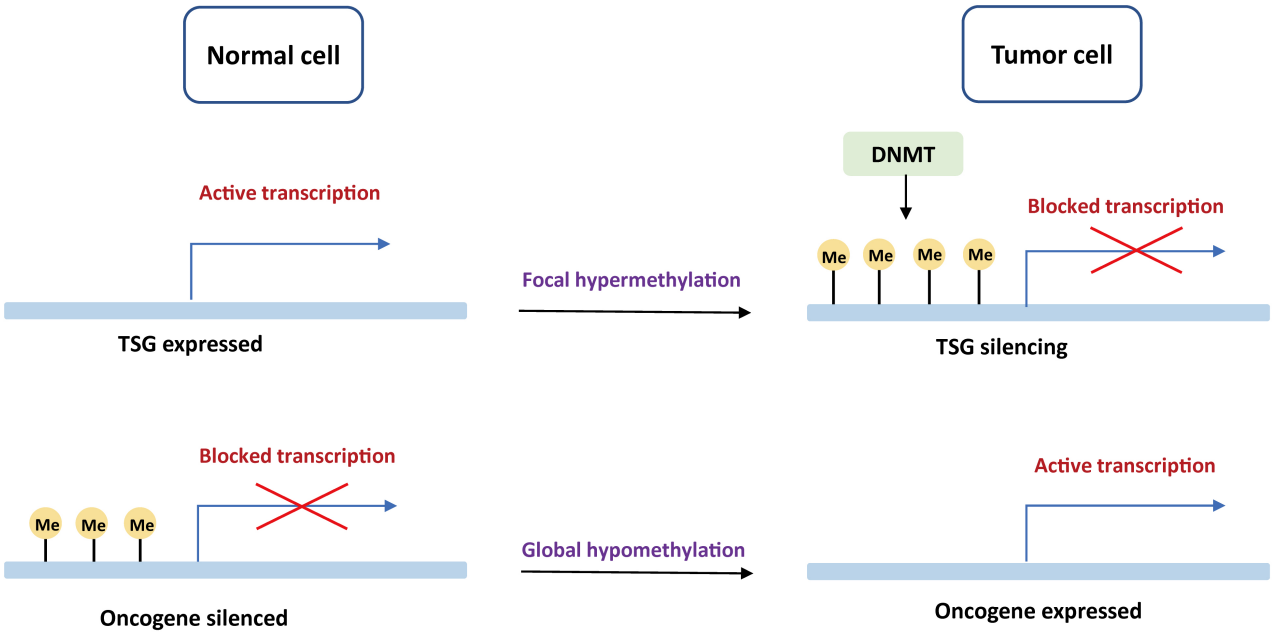


**Fig. 1. Basic mechanisms of oncogenesis induced by DNA methylation.** In normal cells, tumor suppressor genes are demethylated and expressed, while oncogenes are methylated and unexpressed. In the tumor cells, tumor suppressor genes are blocked by DNA de novo methylation with DNMT, whereas oncogenes are actively transcribed due to global hypomethylation. TSG, tumor suppressor gene; DNMT, DNA methyltransferase.

highlight the functions of DNA methylation on immune-oncology and metabolic reprogramming, introduce the emerging strategies to target DNA methylation, and explore potential combinations of epigenetic drugs with other therapies.

## 2. DNA Methylation in Oncogenesis

DNA methylation is the first epigenetic modification observed in human to determine which gene should be turned on or off. Promoter regions contain an increased quality of GC bases that are recognized as CpG islands (*CGI*). Tumors often present a global DNA hypomethylation with gains of focal DNA hypermethylation at CpG-rich sites [15,16]. An aberrant DNA methylation landscape has been reported in a variety of tumors, including both hematological malignancies and solid tumors [17–23]. The mechanisms of carcinogenesis induced by DNA methylation events are displayed in Fig. 1.

### *2.1 Hypomethylation and Gene Activation*

Genome-wide hypomethylation at *CpG* sites has been reported in many tumor types, such as brain tumor, gastric cancer, liver cancer, and breast cancer [24,25]. Though global DNA hypomethylation usually occurs in intergenic regions and contributes less to genetic mutations, it can result in genome instability via gene mutations, deletions, inversions, translocations, and amplifications [26]. For example, Long Interspersed Nucleotide Element 1 (*LINE-1*) is a key component of interspersed DNA repeats. *LINE-1* hypomethylation has been reported in several cancer types and portends to a worse prognosis [27–30]. A recent meta-analysis pointed out that *LINE-1* hypomethylation in tissue samples may serve as an epigenetic marker for cancer risk [30]. In addition, several studies highlighted that hypomethylation of cancer-specific *CGI* may drive the overexpression of oncogenic driver genes [31]. A well-illustrated example is the relationship between *BCL-2* and B-cell chronic lymphocytic leukemia [32].

### *2.2 Hypermethylation and Gene Silencing*

In healthy tissues, the *CpG* dinucleotides at promoter sites of tumor suppressor genes (*TSG*) are generally unmethylated and transcriptionally active. However, approximately 5–10% *CGI* are hypermethylated to prompt *TSG* silencing in cancer cells [33], such as *VHL*, *RB1*, *CDKN2A*, *GATA4*, and *MHL1* [34,35]. DNA hypermethylation was first observed in colon cancer. Studies also indicated that *CGI* methylated phenotype was related to *BRAF* mutations in colon cancer [36–38], whereas glioblastoma displaying promoter hypermethylation was significantly associated with *IDH1* mutations [39,40]. Hypermethylation of mismatch repair gene *MHL1* has been found in human colorectal cancers, which is related to microsatellite instability [41]. Another example is the hypermethylation of *CDKN2A* (*p16*), leading to the inactivation of this gene in esophageal adenocarcinoma [42].

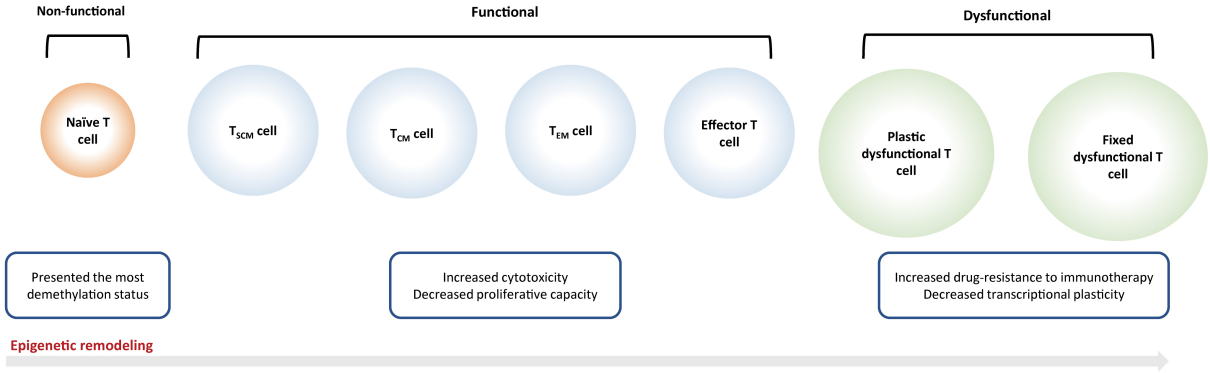


**Fig. 2. Epigenetic reprogramming on T cells.** Naïve T cells will be activated into an 'effector' or 'functional' state after antigen exposure, which is characterized by the rapid secretion of cytokines and effector proteins, as well as the capacity of infiltration and migration into the tumor microenvironment. But continuous antigen stimulation would inevitably result in T cell exhaustion that is subdivided into 'plastic dysfunctional state' and 'fixed dysfunctional state'. $T_{SCM}$, stem cell memory T cells; $T_{CM}$, central memory cells; $T_{EM}$, effector memory T cells.

*2.3 Generation of Tumor Neoantigens*

There is a bidirectional interplay between genetic and epigenetic alterations. In addition to genetic mutations driving the dysregulated epigenetic landscape, DNA methylation-induced mutagenesis are also one of the major sources of genetic alterations, resulting in the formation of tumor-associated neoantigens [43]. DNA methylation enables the cytosine base to be more susceptive to deamination, either spontaneous or mutagen motivated, leading to C to T transitions. DNA hypermethylation of transposable elements is critical to epigenetic silencing, as DNA 5-methycytosine is mostly found to locate in the transposable elements [44]. For example, cancer-testis antigens (CTA) could be suppressed by DNA methylation in most somatic cells, but they can be specifically expressed in normal male germ cells [43]. Importantly, reactivation of CTA-coding genes by demethylation can contribute to the presence of neoantigens with immunogenicity and thus enhance immune surveillance [45].

## 3. Roles of DNA Methylation in the Cancer-Immunity Cycle

Theoretically, the human immune system is able to recognize and eliminate tumor cells involving a series of immune responses, a process called 'cancer-immunity cycle' [46]. It has been reported that tumors commonly hijack various epigenetic remodeling to escape immune surveillance. DNA methylation not only exerts seminal roles in the proliferation and effector functions of $CD8^+$ and $CD4^+$ T cells within the tumor microenvironment (TME) [47], but also profoundly impacts T cell activation and exhaustion (Fig. 2).

*3.1 $CD8^+$ T Cell Fate*

DNA methylation changes can regulate the differentiation of naïve $CD8^+$ T cells into effector T cells, consisting of cytotoxic and memory subtypes [48–51] (Fig. 3). Naïve $CD8^+$ T cells will be activated into an 'effector' state after antigen exposure, which is characterized by the rapid secretion of cytokines and effector proteins, as well as the capacity for infiltration and migration into the TME. However, continuous antigen stimulation would inevitably result in T cell exhaustion that is subdivided into a 'plastic dysfunctional state' and a 'fixed dysfunctional state' [52]. This process is accompanied by an epigenome-wide remodeling wherein hundreds of thousands of genes are variously methylated [53], which are collectively maintained by DNMT1, DNMT3A, and TET2. In the developmental trajectory, genes that prompt the activation, proliferation, and differentiation of T cells are demethylated and highly expressed, such as *IFNG* and *GZMB* [53]. Conversely, unnecessary genes are frequently methylated and regressed, such as *TCF7*. Naïve $CD8^+$ T cells more commonly undergo demethylation compared to cytotoxic and exhausted $CD8^+$ T cells, whereas effector genes of cytotoxic $CD8^+$ T cells generally experienced hypermethylation to hypomethylation from naïve to cytotoxic T cell fate, such as *GZMB*, *IFNG*, *CCL4*, and *CCL3* [54]. Furthermore, memory $CD8^+$ T cells maintain demethylated effector genes, inducing the rapid and robust immune response of memory $CD8^+$ T cells to re-challenge with antigens [52]. DNMT3A is a methyltransferase in charge of de novo methylation. Studies have shown that genetic ablation of DNMT3A may induce fewer effector $CD8^+$ T cells [55]. In addition, the loss of DNMT3A can abolish de novo methylation which serves to establish immunological memory [52].

*3.2 $CD4^+$ T Cell Fate*

Consistently, DNA methylation is responsible for the fate-decision of naïve $CD4^+$ T cells, modulating their differentiation into various effector subtypes, such as Th1, Th2, Th17, Treg, and memory T cells (Fig. 4). Th1 cells

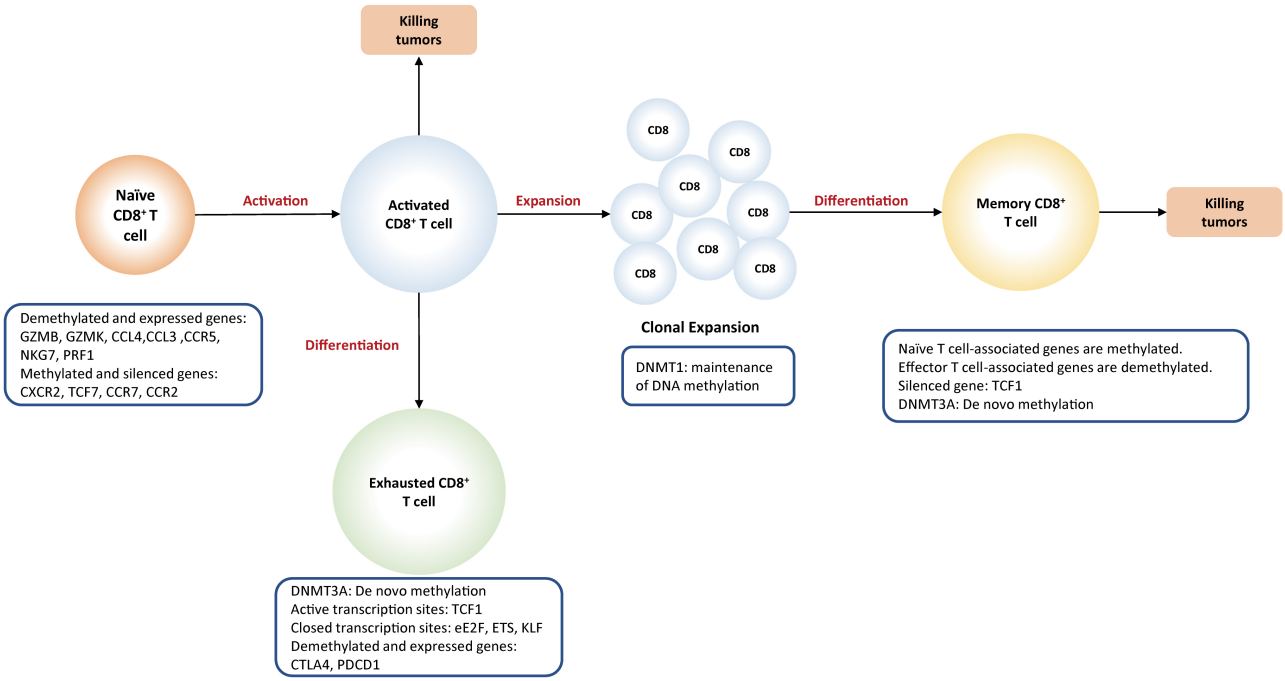


**Fig. 3. DNA methylation plays a key role in the differentiation of CD8$^+$ T cells from naïve to effector status.** DNA methylation changes can lead to the formation of different subtypes of CD8$^+$ T cells, including effector T cells and exhausted T cells. Effector genes of cytotoxic CD8$^+$ T cells generally experienced hypermethylation to hypomethylation from naïve to cytotoxic T cell fate. In memory CD8$^+$ T cells, naïve T cell-associated genes are methylated but effector T cell-associated genes are demethylated. These methylation levels are maintained by DNMT1, DNMT3A, and TET2.

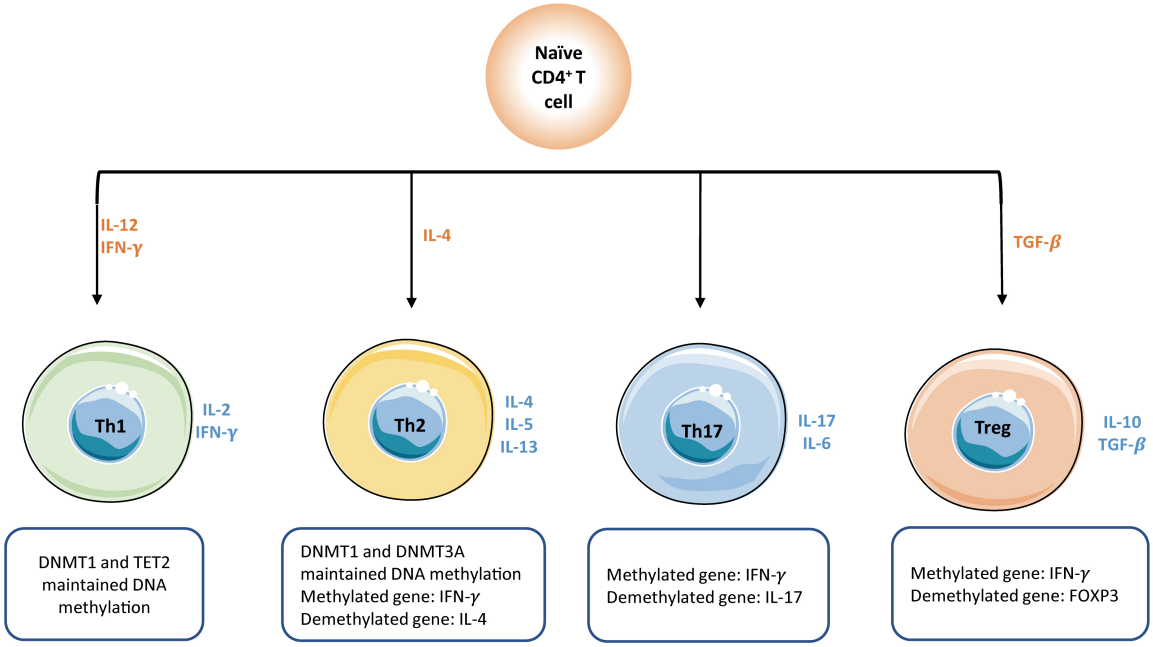


**Fig. 4. DNA methylation plays a key role in the activation of naïve CD4$^+$ T cells into effector T cells, such as Th1, Th2, Th17, and Treg cells.** Naïve CD4$^+$ T cells are stimulated by IL-12 and IFN-$\gamma$ and form Th1 cells, of which process the methylation status is maintained by DNMT1 and TET2. Th1 cells secrete type I cytokines (IL-12 and IFN-$\gamma$) that prompt tumor suppression. The *IFN-$\gamma$* promoter is methylated through de novo methylation catalyzed by DNMT3A during the development of Th2 cells and Th17 cells. Also, the *IL-4* gene is demethylated and upregulated in Th2 cell, while *IL-17* is demethylated and highly expressed in Th17 cell. For the Treg cells, *FOXP3* gene is demethylated at promoters and enhancers, followed by the increased expression of *FOXP3*.

secrete type I cytokines (IL-12 and IFN-$\gamma$) that prompt tumor suppression, whereas Th2 cells secrete type II cytokines (IL-4, IL-5, and IL-13) that polarize immunity towards tumor progression [48,50,51]. The methylation status of immune genes is linked to the immune response in the TME. The differentiation of naïve $CD4^+$ T cells into Th1 and Th2 relies on different epigenetic remodeling of certain genes. It has been demonstrated that some genes present opposite patterns of expression. For example, *IFN-$\gamma$* remains demethylated at promoter regions and upregulates IFN-$\gamma$ production upon Th1 cell differentiation [56]. In contrast, the *IFN-$\gamma$* promoter is methylated through de novo methylation catalyzed by DNMT3A during the development of Th2 cells and Th17 cells [57,58]. Previous studies reported *DNMT3A* deletion may result in the failure of the formation of Th2 and Th17 cells, mainly due to the absence of de novo methylation at the *IFN-$\gamma$* locus [59]. Furthermore, *IL-4* and *IL-17* genes are demethylated and Th2 and Th17 subtypes are activated [59,60]. It has been found that a global loss of DNA methylation occurs in the formation of $CD4^+$ memory T cells [61]. There is, strong evidence to indicate that differentiation of $CD4^+$ naïve T cells into Th1, Th2, Th17, Treg, and memory T cells depends on the dynamic changes of DNA methylation at gene promoter and enhancers, which is maintained by DNMT1, DNMT3A, and TET2.

### *3.3 T Cell Activation*

Activation of naïve T cells initially requires the interactions between T cell receptor (TCR) present on T cells and MHC complex (signal 1) expressed on antigen-presenting cells (APC), followed by the activation of co-stimulatory molecules (signal 2) provided by mature dendritic cells. When both signals are present, T cell activation initiates an autonomous intracellular signaling cascade, leading to T cell expansion and differentiation. Global DNA methylation remodeling plays a critical role in priming and activation of cytotoxic effector T cells. For example, demethylation at a promoter-enhancer region of the *IL-2* loci, significantly drives the increased levels of IL-2 cytokines that are required for T cell activation and proliferation [62]. Promoter demethylation is observed in several effector genes, such as *GZMK* and *GZMB* [53].

### *3.4 T Cell Exhaustion*

T cells become dysfunctional or exhausted when they are exposed to continuous antigen stimulation, resulting in the failure to produce effective immune response. Generally, exhausted T cells display high levels of inhibitory receptors, such as PD-L1, leading to reduced effector function and suppressed T cell proliferation [63–65]. It is recognized that DNA methylation contributes to regulating *PD-L1* expression. Compelling evidence implicates that *PD-L1* promoter is demethylated within exhausted $CD8^+$ T cells in multiple tumors [66–68], whereas PD-L1 in effector $CD8^+$ T cells remained in the methylated status. PD-1 blockade has the ability to reverse T cell exhaustion in both chronic infection and tumor settings. But this reinvigoration is transient, as T cells become re-exhausted after the withdrawal of PD-1 blockade [69]. Based on ATAC-seq analysis, this is mainly because immune checkpoint inhibitor (ICI) is unable to completely revert exhausted T cells into effector T cells [70]. Furthermore, de novo DNA methylation by DNMT3A at post-effector stage is required for the exhaustion phenotype [70]. Genetic ablation of *DNMT3A* significantly induced the generation of effector cytokines in the mice model infected with lymphocytic choriomeningitis virus (LCMV) [70]. PD-1 blockade does not erase exhaustion-related DNA methylation. The ablation of *DNMT3A* combined with a DNA demethylating agent before PD-1 blockade, will result in pronounced T cell expansion and effective immune responses. Overall, aberrant DNA methylation at specific gene loci confers T cell exhaustion [54,63,71]. ICI can successfully rejuvenate exhausted T cells that have been epigenetically remodeled.

## 4. Roles of DNA Methylation on Metabolism Reprogramming

Dysregulated metabolism represents a hallmark of malignancy [72,73]. Recently, accumulating evidence suggests that DNA methylation affects malignant cells' metabolism and vice versa [13]. The metabolic pathways that DNA methylation participates in mainly involve glycolysis, methionine cycle, and tricarboxylic acid (TCA) cycle [74–76] (Fig. 5).

DNA methylation indirectly controls the preferential use of aerobic glycolysis (the Warburg effect) even with an abundant supply of oxygen, contributing to a higher glycolytic influx. Several *TSGs* that regulate aerobic glycolysis are silenced by DNA hypermethylation, such as *VHL* which is crucial to *TSG* in the HIF pathway [77]. Therefore, *VHL* deficiency reinforces glycolytic activity [78]. Also, studies have demonstrated that hypermethylation of the *VHL* promoter induces gene silencing and consequently reduces HIF1-mediated proteolysis in multiple myeloma [77]. Additionally, promoter demethylation is associated with overexpression of *HK2* and *PKM2*, both of which favor enhanced glycolysis [79–81]. Oppositely, promoter hypermethylation leads to the gene silencing of *FBP-1* and *FBP-2* that encode rate-limiting enzymes in glucogenesis. The silence of *FBP-1* and *FBP-2* could limit gluconeogenesis but support glycolysis, which is beneficial for the proliferation of tumor cells [82,83].

The methionine cycle gives rise to SAM which is a methyl donor required by DNMT to methylate DNA. It is thought that navigating the methionine cycle may counter leukemogenesis, especially for *DNMT3A*-mutated AML [84]. Authors have confirmed the findings that alteration of SAM metabolism and *DOTL1* inhibition exert a synergistical effect on inhibiting leukemogenesis [84,85].

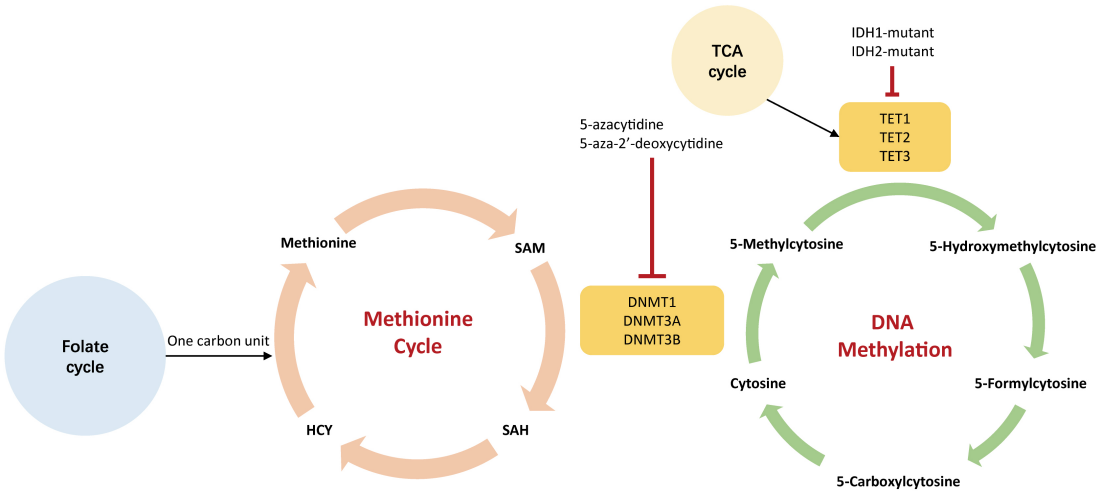


**Fig. 5. The association between DNA methylation and metabolism.** The green cycle represents DNA methylation that occurs on genome; the orange cycle indicates methionine cycle that generates SAM in the cytoplasm. The 5-carbonxylcytosine could be methylated to form 5-methylcytosine by DNA methyltransferases (DNMTs), including DNMT1, DNMT3A, and DNMT3B. Small molecules agents, such as 5-azacytidine and 5-aza-2'-deoxycytidine, could inhibit the activity of DNMTs leading to hypomethylation. These hypomethylating agents are used to treat a variety of human tumors. IDH1-2, isocitrate dehydrogenase 1-2; HCY, homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DNMT, DNA methyltransferase; TET, ten-eleven translocation demethylase.

Epigenetic enzymes require TCA metabolites as either substrates or co-factors for post-translational modifications of DNA. For example, the TET family (TET1, TET2, and TET3) is a group of DNA demethylases that are flavin adenine dinucleotide (FAD)- and $\alpha$-ketoglutarate ($\alpha$-KG)-dependent. Both FAD and $\alpha$KG are generated in the TCA cycle [86–88]. Likewise, mutant metabolic enzymes that are involved in the TCA cycle might facilitate aberrant DNA methylation as well. Distortion of succinate dehydrase (SH) and fumarate hydratase (FH) would lead to the accumulation of succinate and fumarate, which in turn induces DNA hypermethylation by interfering the TET functions [89–92]. Similarly, $NADP^+$-dependent isocitrate dehydrogenase (IDH1/2) mutations lead to a high concentration of D2HG that would inhibit the activity of TET-family DNA demethylases [93].

Metabolism not only orchestrates epigenetic marks but also functions in cancer immunity [94,95]. Several studies supported that metabolism reprogramming impacts intercellular signaling cascade and epigenetic landscape to regulate the longevity and functionality of T cells [96]. Both epigenetics and metabolism are able to regulate T cell exhaustion [97]. Specifically, exhausted T cells would go through nutrient deficiency, along with the altered epigenome.

## 5. Modulators of DNA Methylation

Abnormalities in DNA methylation are related to tumor initiation and progression [98]. Therefore, a large number of epigenetic modulators are currently being developed to target DNA hyper- and hypo-methylation (Table 1). Several trials are ongoing to investigate potential combinations of therapies [15,99]. Epigenetic modulators can be broadly divided into 'reprogramming agents' and 'targeted agents'. The former includes DNMT inhibitors (DNMTi) that may reverse abnormal DNA methylation patterns, whereas the latter specifically targets genetic alterations in the epigenetic pathways, such as targeting *IDH1/2* mutations in AML.

### *5.1 DNMT Inhibitors*

*TSG* silencing caused by promoter hypermethylation is a common mechanism of tumorigenesis, which greatly facilitated the discovery of DNA methyltransferase inhibitors (DNMTi) in the past few decades [100–103]. DNMTi, also termed as hypomethylating agents, are the most widely utilized epigenetic drugs, especially for treating hematologic malignancies. Analogues of the nucleoside cytidine involve 5-azacytidine (5-AZA), 5-aza-2'deoxycytidine (decitabine), and SGI-110 (guadecitabine), which make DNMT inaccessible to DNA and consequently cause DNA hypomethylation [104–106]. Both 5-AZA and decitabine have been approved by the Food and Drug Administration (FDA) [107]. These two drugs are currently used as first-line therapy for myelodysplastic syndrome (MDS), bringing improved response rates and prolonged survival [108,109]. Impressive success has also been achieved in the treatment of acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML) (Table 1). In addition, DNMTi has shown improved outcomes in solid tumors, such as ovarian cancer and non-small cell lung cancer [110–112].

**Table 1. Genetic alterations of CMP and small molecular inhibitors of CMP for cancer.**

| | Target | Genetic alteration | Inhibitors | Status | Indication |
|---|---|---|---|---|---|
| DNA methylation | DNMT | *LOF*-mutations; Dominant-negative mutations at *Arg882* | Decitabine | Approved | MDS, AML |
| | | | Azaciditine | Approved | MDS, AML |
| | | | Guadecitabine (SGI-110) | Phase-III | MDS, AML, CMML |
| | TET2 | *LOF*-mutations | NA | NA | NA |

Abbreviations: CMP, chromatin-modifying protein; DNMT, DNA methyltransferase; TET, ten-eleven translocation dioxygenase; LOF, loss-of-function; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; NA, not available.

### *5.2 TET Inhibitors*

There is a frequently overlooked phenomenon that the genome-wide DNA hypomethylation occurs in several solid tumors [113], which is related to tumor initiation and progression. *TET2* mutations are detected in approximately 20–25% of MDS, 7–23% AML, and over 53% CMML [114–116]. Further, previous studies indicated that *TET1* acts as a suppressor of hematological malignancies [117,118]. However, there are no approved TET inhibitors to reverse DNA hypomethylation, though several agents are being tested at preclinical stages. For example, the novel compound Bobcat339 has been shown to directly block the enzymatic activity of TET1/2 [119]. Since the *JAK/STAT* pathways are involved in TET1 transcription, STAT inhibitor UC-51423 may avoid aberrant TET1 function [120]. An alternative approach is to target SAM that donates the methyl group and manipulates the level of methylation. More importantly, emerging studies suggest that SAM could modulate cancer immunity by affecting the functionality of $CD8^+$ T cells [121].

## 6. Multiple Drug Combinations.

### *6.1 Combinations of DNMTi and HDACi*

Since DNA methylation and histone modification usually work in parallel, considerable efforts are being made to explore various combinations that may significantly increase the efficacy of a single agent and decrease drug resistance. For instance, in a murine ovarian cancer model, the combination of DNMTi and histone deacetylase (HDACi) is capable of increasing the cytotoxic activity of $CD8^+$ cells and NK cells, and promoting effector T cell infiltration in the tumor microenvironment (TME) [122]. Similarly, as the location of DNA methylation and enhancer of zeste homolog 2 (EZH2)-mediated histone acetylation in chromatin are usually mutually exclusive, EZH2 is able to maintain quiescence of particular genes when CGI become activated due to DNA hypermethylation [123,124]. Therefore, the combination of DNMTi with EZH2 inhibitors is a promising method to enhance anti-tumor responses.

### *6.2 Combinations of DNMTi and ICI*

Numerous clinical trials are evaluating the combination of epigenetic modulators with immune checkpoint inhibitors (ICIs) [125]. The rationale for this proposal is that epigenetic drugs may assist immunotherapy to increase the ability of cytotoxic T cells to attack malignant cells [126–129]. ICI, an exciting cancer therapy which has emerged in the past several years, has been approved by FDA for non-small cell lung cancer (NSCLC) , melanoma, and renal cancer [130–132]. There was an unexpected finding that a group of advanced NSCLC patients who had relapsed after a combination of azacytidine (DNMTi) and entinostat (HDACi), received a robust and durable response when they were subsequently enrolled in a clinical trial of ICI [129]. However, it is unknown whether the favorable outcomes reflect the effectiveness of combined therapy or are merely responses to the exposure to ICI. Numerous efforts have been made to figure out the underlying mechanisms as to how epigenetic therapy mediates the augmentation of ICI. It is thought that DNMT inhibitors could reverse immune evasion, as they are able to upregulate the expression of tumor-associated antigens (TAAs) and MHC molecules on the tumor cell surface [133,134]. Recent studies highlighted that 'viral mimicry' produced by DNMTi was tightly associated with increased interferon that may trigger immune attraction [126,127]. Interests have been switched into the activation of endogenous retroviruses (ERV) which are generally methylated and suppressed in most somatic cells. Mounting evidence suggests that ERV expressed in clear cell renal cell carcinoma could encode peptides that induce T cell and B cell immunoreactivity [135]. As cytosine methylation is critical to the regulation of ERV, reactivation of ERV could be achieved by exposure to DNMTi, and might be effective in reversing immune tolerance for ICI therapy [126,127,136,137].

### *6.3 Combinations of DNMTi and Metabolic Modulators*

Aberrant DNA methylation is an epigenetic memory signature that is central to the pathobiological of *IDH*-mutant tumors, such as gliomas and AML [138]. DNMTi could be used in combination with isocitrate dehydrogenase (IDH) inhibitors, reducing the production of oncometabolites. More specifically, inhibition of mutant forms of IDH, an enzyme in the TCA cycle, could impact the demethylation status of DNA and histone. Findings also demonstrated that DNMTi might be more effective than IDH inhibitors [139], but the efficacy of combination therapy is still un-

known. Another example is the combination use of DNMTi with serine metabolism that has been shown to more aggressively kill liver kinase B1-deficiency tumors carrying *KRAS* [140]. Overall, despite great attentions in targeting both metabolism reprogramming and epigenetic remodeling, it is unknown whether these two hallmarks function in a synergistical manner.

### *6.4 Combinations of DNMTi and Chemotherapy*

Preclinical studies suggest that DNMTi may improve outcomes when combined with standard cytotoxic drugs. Epigenetic regulation might be a potential mechanism leading to drug resistance to cytotoxic drugs, and thus DNMTi could reverse these pathways and improve the durability of clinical responses [141]. One ongoing clinical trial is being tested to combine DNMTi with cytotoxic agents, making ovarian cancers re-sensitive to these standard drugs [142–146]. Furthermore, a series of clinical trials are underway to explore combinations of DNMTi with chemotherapy, in an attempt to restore chemosensitivity among patients whose disease have relapsed.

### *6.5 Triple Combination*

A triple combination of DNMTi, HDACi, and ICI, has also been shown to trigger a stronger anti-tumor effect. The application of ICI following combined inhibition of DNMT1 and EZH2 would make ovarian cancer cells to express *CXCL9* and *CXCL10*, which could activate T helper 1 cells and attract tumor-infiltrating lymphocytes (TILs) [147].

## 7. DNA Methylation in Cancer Prevention

There is a remarkable association between epigenetics and environmental exposure. Lifestyle factors, such as diet, body weight, smoking, and physical activity, constitute the majority of cancer causes, playing an essential role in cancer prevention [148]. One research put forward that lifestyle may modify DNA methylation status leading to genome reprogramming [149]. For example, a study illustrated that the intake of 'healthy food' is positively associated with *LINE-1* methylation level, which was negatively related to the genome instability [150]. Besides, it has been proven that increased BMI was associated with a lower *LINE-1* methylation, particularly for obese females [151]. Increasing evidence suggested that nutritional status may have a lasting effect on metabolism via DNA methylation. A prospective study found that higher dietary folate intake was associated with less *LINE-1* hypomethylation in colon cancer, whereas higher alcohol consumption is related to a higher risk of *LINE-1* hypomethylation [152]. It is notable that the degree of *LINE-1* hypomethylation had a dose-response relationship with poor prognosis and higher mortality in colon tumors [153]. Therefore, modulating DNA methylation may hold the potential to reverse the harmful effects of dietary and lifestyle on tumorigenesis.

## 8. Conclusions

In summary, both hypomethylation and hypermethylation of DNA coexist in the process of oncogenesis. DNA hypomethylation is capable of activating proto-oncogenes that leads to genomic instability, while DNA hypermethylation could silence the promoters of *TSGs* that are frequently associated with tumor suppression. Epigenetic alterations may reactivate several genes that are normally limited to immune-privileged organs, such as *CTA*. These neoantigens are immunogenic and thus enhance the visibility to immune surveillance. In addition, DNA methylation plays critical roles in the regulation of metabolic reprogramming and the cancer-immunity cycle, and thus intensive investigations have focused on expanding the application of DNMTi to rational combinations with other cancer treatments. The efficacy of single DNMTi is likely to be enhanced by combinatorial therapies. Combining DNA methylation inhibitors with ICIs might sensitize less-immunogenic tumors and overcome immune escape. Combing DNMTi and HDACi can synergistically increase the expression levels of *TSG*. A combination of DNMTi with chemotherapy may restore sensitivity and reverse drug resistance in relapsed patients. Finally, the reciprocal connection between metabolism and epigenetics will continue to motivate this novel combination strategy. Identifying the most effective epigenetic targeting strategies and exploring rationale-based combinational strategies to boost durable anti-tumor response will play an important role in future clinical practice.

## Author Contributions

YQ designed the study and reviewed the manuscript. CC and ZW participated in study design and wrote the original draft of the manuscript. CC was mainly responsible for the design of tables and figures. YD, LW, SW, and HW contributed to the conception of the paper. All authors agreed to the submission of the final manuscript.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflict of Interest

The authors declare no conflict of interest.

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