



## EDITORIAL

# DNA methylation profiles in cancer: functions, therapy, and beyond

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## DNA methylation in cancer

DNA methylation, a reversible post-replication modification, defines gene expression and function in different physiologic processes. Hypomethylation is quite common among global DNA modifications, while promoter hypermethylation often occurs in local regions during cancer, as in CpG island formation, thereby perpetuating the inactivation of tumor suppressor genes. Additionally, CpG islands undergo remethylation during embryonic development after demethylation by germ cells. It is widely accepted that gene promoters, particularly those of essential tumor suppressor genes, remain unmethylated in healthy tissues and become substantially methylated in cancerous tissues<sup>1</sup>. Therefore, inappropriate methylation of DNA after remethylation can lead to a variety of diseases, including progressive inflammatory diseases, precancerous lesions, and cancers.

Abnormal DNA methylation is often found in cancer and is considered an important indicator of malignant progression. This phenomenon is also commonly detected in malignant tumors of the blood system, including leukemias, lymphomas, and multiple myeloma, which manifest as hypomethylation of the entire genome and abnormal hypermethylation

of CpG islands. This finding usually results in silencing of tumor suppressor genes and overexpression of oncogenes. Alterations in DNA methylation in hematologic malignancies typically involve decreased methylation levels in the methyltransferase (DNMT1, DNMT3A, and DNMT3B) gene promoter CpG island or mutations in the methyltransferases or demethylase genes, as proposed by Zhao et al.<sup>2</sup> DNA methyltransferases (DNMTs) directly catalyze DNA methylation by DNMT1 maintaining existing methylation and DNMT3A and DNMT3B catalyzing *de novo* methylation.

## DNA methylation as a cancer biomarker

Abnormal DNA methylation is a hallmark of tumorigenesis and cancer development<sup>3</sup>. Dysregulated DNA methylation is frequently observed in the promoter region and dysregulated DNA methylation silences the expression of tumor suppressor genes or removes restrictions on oncogenes. Widespread changes in DNA methylation typically occur in the early stages of tumor development, indicating the important role of abnormal DNA methylation. Identifying the functional consequences of abnormal DNA methylation is therefore crucial for improving tumor diagnosis, prognosis, and treatment<sup>4,5</sup>. However, identifying DNA methylation patterns that function in tumor formation and distinguishing DNA methylation patterns from tissue-specific epigenetic features remains a challenge. Although some analytical methods have been used to characterize the relationships between DNA methylation and gene expression changes, most studies have only focused on transcriptome expression.

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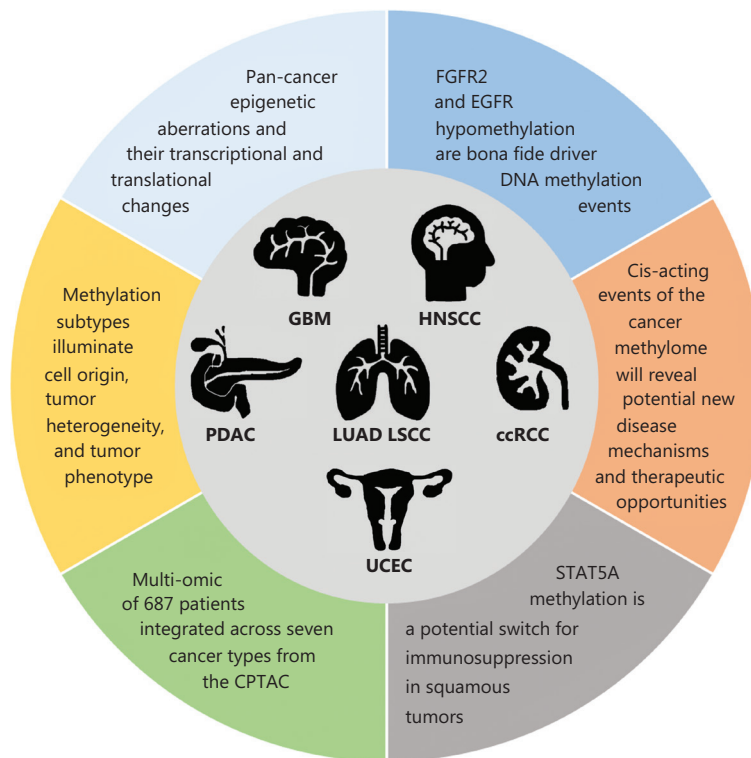
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## Integrative multi-omic cancer profiling has revealed abnormal DNA methylation patterns and unique epigenetic characteristics in seven tumor types

Genome-scale and RNA-seq analyses generally lack a comprehensive and direct demonstration of the interaction and regulation between molecular targets in different malignancies. Therefore, researchers involved in the Clinical Proteomic Tumor Analysis Consortium have analyzed various proteins and the associated abnormal DNA methylations that drive cancer in pan-cancer studies. Liang et al.<sup>6</sup> evaluated the crucial role of dysregulated DNA methylation in establishing and maintaining cellular characteristics, and in the development of tumors. To identify aberrant methylations associated with RNA and protein abundance changes, Liang et al.<sup>6</sup> conducted

an analysis of DNA methylation patterns combined with RNA array and proteomic analyses based on multi-omic profiling of 687 tumors and matched normal adjacent tissues from the kidneys, brain, pancreas, lungs, head, endometrium, and neck. By constructing a Pan-Cancer catalog, the authors identified lineage-specific epigenetic drivers, including hypomethylated *FGFR2* in endometrial cancer, and further showed that hypermethylated *STAT5A* was associated with pervasive regulon downregulation and immune cell depletion. Overall, Liang et al.<sup>6</sup> identified cis-acting DNA methylation events that drive transcription and translation changes, revealing the epigenetic landscape of tumors and the role of the originating cells. Liang et al.<sup>6</sup> further reported DNA methylation-mediated tumorigenesis and provided insight into the development of epigenetic therapies during DNA methylation (Figure 1). Based on pan-cancer DNA methylation patterns, investigators have suggested that by analyzing and determining DNA methylation isoforms and immune molecule epigenetic signature clusters,



**Figure 1** Integrative multi-omic cancer profiling reveals abnormal DNA methylation patterns that promote cancer and identify links to tumor immunotherapy. GBM, glioblastoma multiforme; HNSCC, head and neck squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; LUAD, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; ccRCC, clear cell renal cell carcinoma; UCEC, uterine corpus endometrial carcinoma.

it may be possible to personalize an individual patient's treatment by targeting different DNA methylations, and consequently the immune proteomics target molecules.

## **DNMT overexpression frequently contributes to the progression of hematologic malignancies**

DNMT1 alterations have been frequently observed in leukemias and lymphomas, and increased DNMT1 expression has been shown to lead to the development of leukemias by causing abnormal regional hypermethylation. In the treatment of hematologic malignancies, DNMT1 overexpression is related to advanced clinical staging and treatment resistance in diffuse large B-cell lymphomas<sup>7</sup>. Conditional knockdown of DNMT1 suppresses leukemogenesis, while in lymphomas, DNMT1 plays a critical role in the maintenance of MYC-induced T-cell lymphomas and Burkitt's lymphomas (BLs). DNMT3A mutations are frequently detected in all forms of leukemia, including acute myeloid leukemia (AML), where they are among the most prevalent genetic alterations. These mutations are associated with various clinical features of leukemia. Specifically, there is overexpression of DNMT3A protein in AML and during the acute phase of chronic myeloid leukemia (CML). This finding indicates that the level of DNMT3A expression potentially serves as a prognostic biomarker. Additionally, studies have suggested that when combined with other mutations DNMT3A mutations result in unfavorable prognoses for patients with leukemia. Aberrant methylation due to DNMT3B is associated with the development of hematologic malignancies. Furthermore, overexpression of DNMT3B has been demonstrated in AML and CML patients. Overexpression of DNMT3B has been characterized as a negative prognostic factor in patients with BL.

## **Overexpression of DNMTs increases the potential for solid tumor formation**

DNMTs, such as DNMT1, have bilateral roles in other types of tumors, in which DNMT1 drives malignant progression. Zagorac et al.<sup>8</sup> showed that DNMT1 is specifically upregulated in pancreatic ductal adenocarcinoma (PDAC) by examining genome-wide DNA methylation profiles of PDAC cancer stem cells (CSCs). In fact, some CpG islands that were normally

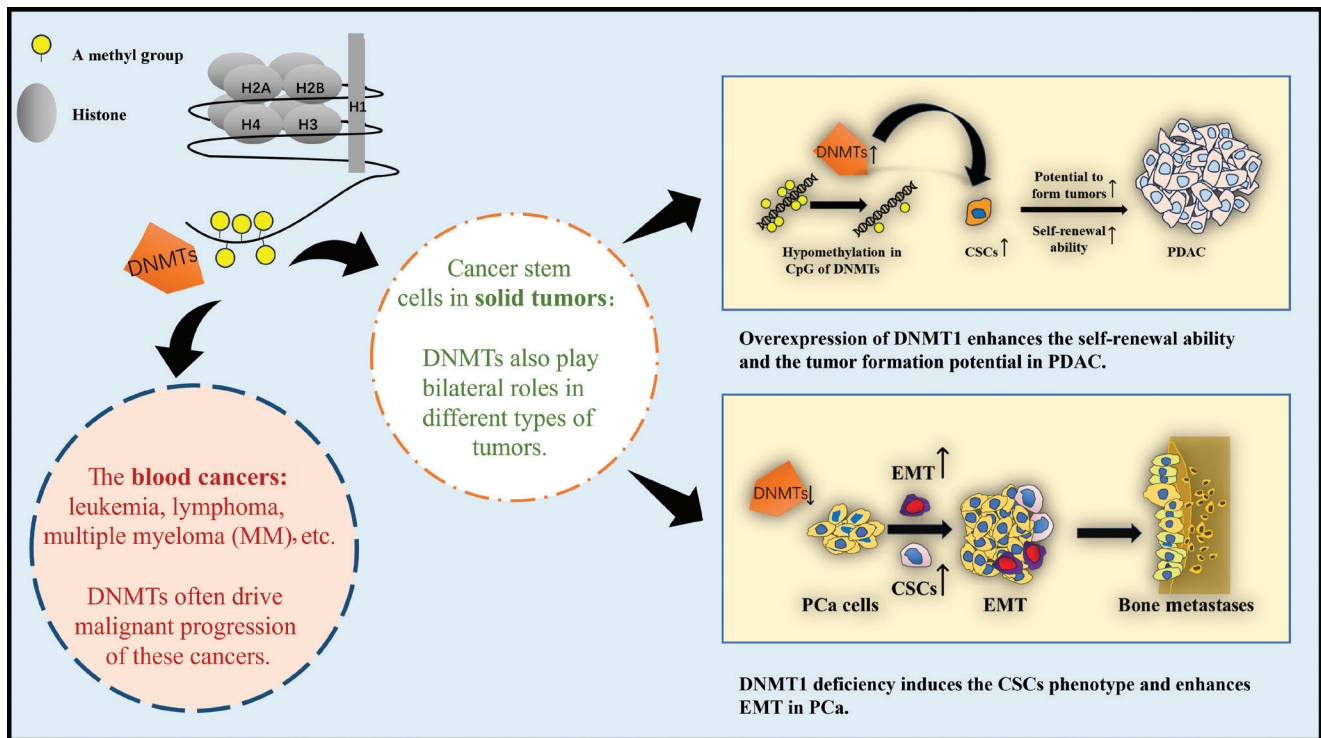
methylated in normal tissues were hypomethylated and actively transcribed in cancer cells. Based on this observation, it was suggested that PDAC CSCs may defend the genome against undesirable transcription or instability, resulting in enhanced survival and growth fitness, when compared to less specialized non-CSC equivalents. In addition, pharmacologic or genetic targeting of DNMT1 in CSCs has been shown to reduce the self-renewal ability and potential to form tumors in PDAC.

## **DNMT deficiency induces the CSC phenotype and enhances the epithelial-mesenchymal transition (EMT) in solid tumors**

DNMT1 deficiency in some tumors tends to induce the CSC phenotype and enhance the EMT. Most prostate cancer (PCa) patients die after bone metastasis, which is associated with the induction of the EMT and CSCs. Lee et al.<sup>9</sup> reported that repression of DNMT1 by reducing DNMT1 expression in PCa cells with 5-Aza, a DNMT1 inhibitor, leads to induction of the EMT as well as the formation of CSCs *in vitro*. Furthermore, siRNA-mediated DNMT1 silencing also induces the expression of the EMT and CSC malignant phenotypes in PCa cells. Mechanistically, the reduction of DNMT1 may lead to repression of *H3K9me3* and *H3K27me3* on the promoter regions of *Zeb2* and *KLF4* in PCa cells. In an animal model, PCa cells injected after pretreatment with 5-Aza resulted in significantly larger tumors when compared with PCa cells injected without pretreatment. Additionally, pretreated tumor cells exhibited increased spread in bone tissue. This dual nature of DNMTs in various tumor types suggests the possibility of targeting DNA methylation for therapeutic purposes (Figure 2).

## **DNA methylation inhibition is used for targeted therapy in preclinical cancer treatments**

Abnormal DNA methylation is linked with the development of numerous diseases, including cancer, so it is important to develop treatments to correct imbalances in abnormal methylation<sup>10,11</sup>. Based on the complexity of cancer, DNA methylation changes have wide-ranging effects on the disease landscape, and molecular-targeted treatment of methylation



**Figure 2** DNA methyltransferases (DNMTs) have various roles in the progression of different types of tumors. DNMTs tend to promote malignant progression of hematologic tumors and have a bilateral role in solid tumors. For example, the CpG islands of DNMT1 show hypomethylation in pancreatic ductal adenocarcinoma (PDAC) cancer stem cells (CSCs). DNMT overexpression enhances self-renewal ability and the tumor formation potential in PDAC. Conversely, DNMT deficiency has been shown to induce the CSC phenotype, strengthen the epithelial-mesenchymal transition (EMT), and result in bone metastasis in prostate cancer (PCa).

abnormalities has become an effective way to treat cancer. Furthermore, the phenotypic and functional heterogeneity of human cancers have been conclusively demonstrated. The variability in tumor epigenomics results in substantial differences between distinct subsets of tumor cells<sup>12</sup>. Identifying key methylation changes in various cancers through pan-cancer analyses is therefore crucial in identifying relevant molecular targets associated with these abnormalities.

Chimeric antigen receptor T (CAR-T) cell therapies that are effective against hematologic tumors frequently show restricted efficacy in solid tumors, which is attributed to limited persistence, restricted trafficking, and reduced tumor infiltration. Like CAR-T therapies, epigenetic therapies have achieved impressive results in hematologic malignancies but have been less effective for some solid tumors that are sensitive to immunotherapy. In a genome-wide analysis of single-cell DNA methylation sequencing, Guo et al.<sup>13</sup> discovered clusters of anti-tumor immune gene-enriched loci that were silenced and retained their methylation in long-range hypomethylated

regions of CTCs and early PCa, suggesting that early epigenetic alterations may influence tumorigenesis. Early hypomethylation of partially methylated domains in the core of PCa tumors can cause differential suppression of genes related to immune surveillance, while preserving genes that facilitate cell proliferation, indicating the necessity of determining the potential of combination therapy using epigenetic and immunotherapies. Therefore, combining DNA methylation targeting and immune therapy will be an important direction for future tumor treatment development.

## Inhibition of DNA methylation enhances intrinsic immune responses and immune cell functions during tumor progression

Chronic infections and cancers stimulate T cells, leading to a state of exhaustion, which is characterized by inadequate

secretion of effector cytokines, limited proliferative capacity and persistence, and expression of inhibitory surface receptors. These events collectively reduce the effectiveness of T cell-mediated immunity. However, according to studies by Belk et al.<sup>14</sup>, the epigenetic state of T-ex cells is remarkably stable in exhausted T cells (T-ex), indicating that extended exposure to antigens has a lasting impact that cannot be removed *via* discontinuation of antigen exposure or PD-1 blockade. The results also suggest that this effect may be attributable to DNMT3A, a DNMT that mediates remethylation of DNA during embryogenesis. The remethylation process remains unaffected by PD-L1 blockade and combining anti-PD-L1 blockade therapy with DNMT3A inhibition enhances T cell proliferation during chronic infections and improve treatments. Furthermore, DNMT3A knockdown in CAR-T cells has shown increased anti-tumor activity, better proliferation, and enhanced effector functions, while also exhibiting reduced T cell exhaustion.

DNMTs also have an important dual role in tumor drug resistance. For example, treatment with decitabine, a small molecule inhibitor of DNMT1, increases the sensitivity of pancreatic cancer cells to sorafenib, and inhibition of DNMT3B with nanaomycin A also significantly increases the sensitivity of hepatocellular carcinoma (HCC) cells to sorafenib<sup>15</sup>. However, decitabine sensitivity is closely associated with high expression of DNMT1 in ovarian cancer<sup>16</sup>, which suggests the significance of individualizing treatments for different patients by classifying pan-cancer DNA methylation isoform clusters<sup>17</sup>. In terms of novel treatment strategies, DNMT inhibitors are receiving increasing attention in cancer therapy and have significant potential for treating various types of cancers. Current research is therefore aimed to develop more precise DNMT inhibitors to selectively inhibit DNA methylation in cancer cells without affecting healthy cells. Furthermore, survival of patients with various types of cancer can be improved using tailored treatment approaches and enhanced drug delivery systems<sup>18</sup>.

## Conclusions

Recently, researchers have demonstrated that DNA methylation has a crucial role in tumorigenesis, controlling the cellular phenotype, and regulating gene expression in all types of cancers. Abnormal DNA methylation and associated methyltransferases have been extensively studied in leukemia and CSCs of solid tumors and have shown promising cancer targeting

potential. Different DNA methylation isoforms with potential therapeutic and prognostic values have been identified with respect to RNA and protein features, and the methylome is stratified by immunologically relevant targets that co-occur against tumor subpopulations in different organs, thus improving the efficacy of immunotherapy in cancer patients. For ideal current tumor therapy and epigenetic therapies targeting abnormal DNA methylation, a combination of multi-omics pan-cancer analyses can identify distinct clusters of aberrant DNA methylation isoforms and inhibitory targets for immunotherapy to guide personalized clinical treatments.

There are several limitations in these findings. First, reports have primarily focused on DNA methylation occurring in cis-acting promoter regions. Studies involving DNA trans-acting methylation within and between genes are lacking. Second, DNA methylation function that go beyond regulating gene expression have not been studied. The functions may include the transcriptional effects on tumor cells, rather than the actual transcriptional state, and may also influence the epigenetic plasticity of tumor cells. Finally, comprehensive multi-omics analyses have been limited in the ability to differentiate DNA methylation changes in cancer epithelial cells or tumor-infiltrating lymphocytes. It is also critical to evaluate the significance of such changes in these and other healthy tissues to identify significant therapeutic treatments.

Although incorporating transcriptional profiling in the evaluation of aberrant DNA methylation can improve the characteristics of molecular tumors to select specific cancer treatment methods, this cannot reliably predict changes in the corresponding protein levels or functional states targeted by most anticancer drugs. Li et al.<sup>19</sup> conducted multi-omics pan-cancer analyses and reported the impact of cancer drivers by quantifying significant cis effects and distal trans effects at the RNA, protein, and phosphoprotein levels. In addition, Geffen et al.<sup>20</sup> analyzed a large proteomics-genomics dataset from 1,110 posttranslational modification (PTM) patients with 11 cancer types. The study revealed pan-cancer patterns of protein acetylation and phosphorylation involved in cancer, which highlighted the rich biology governed by PTMs and identified potential new therapeutic avenues. Most common tumors have many mutations of unknown significance, which makes it difficult to determine the abnormalities that are important oncologic drivers. A combination of proteomics and genomics can therefore provide a better understanding of the molecular composition of individual patients and their tumors, which will help identify potential therapeutic

molecular targets and make it possible to study the relationships between molecular discoveries and cancer treatment outcomes to further accelerate new clinical trials using biomarkers with prognostic and predictive values.

## Author contributions

Writing original draft: Jinrong Zhu, Rongxin Zhang.  
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 All authors read and approved the final manuscript.

## Conflict of interest statement

No potential conflicts of interest are disclosed.

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