Published online 1 December 2021

NAR Cancer, 2021, Vol. 3, No. 4 **1**
https://doi.org/10.1093/narcan/zcab045

SURVEY AND SUMMARY

Misregulation of the expression and activity of DNA methyltransferases in cancer

Isaiah K. Mensah[1], Allison B. Norvil[2], Lama AlAbdi[3], Sarah McGovern[1], Christopher J. Petell[4], Ming He[1] and Humaira Gowher[1,*]

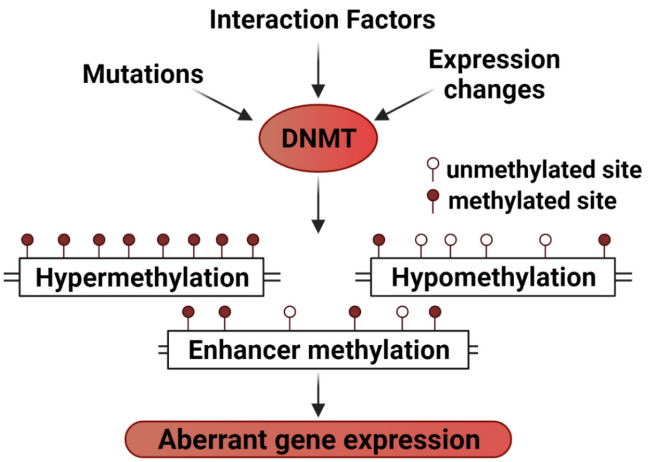
[1]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA, [2]Eli Lilly & Co, IN, USA, [3]Department of Zoology, Collage of Science, King Saud University, Riyadh, Saudi Arabia and [4]Cook Research Institute, West Lafayette, IN, USA

Received September 20, 2021; Revised October 29, 2021; Editorial Decision November 09, 2021; Accepted November 10, 2021

ABSTRACT

In mammals, DNA methyltransferases DNMT1 and DNMT3's (A, B and L) deposit and maintain DNA methylation in dividing and nondividing cells. Although these enzymes have an unremarkable DNA sequence specificity (CpG), their regional specificity is regulated by interactions with various protein factors, chromatin modifiers, and post-translational modifications of histones. Changes in the DNMT expression or interacting partners affect DNA methylation patterns. Consequently, the acquired gene expression may increase the proliferative potential of cells, often concomitant with loss of cell identity as found in cancer. Aberrant DNA methylation, including hypermethylation and hypomethylation at various genomic regions, therefore, is a hallmark of most cancers. Additionally, somatic mutations in DNMTs that affect catalytic activity were mapped in Acute Myeloid Leukemia cancer cells. Despite being very effective in some cancers, the clinically approved DNMT inhibitors lack specificity, which could result in a wide range of deleterious effects. Elucidating distinct molecular mechanisms of DNMTs will facilitate the discovery of alternative cancer therapeutic targets. This review is focused on: (i) the structure and characteristics of DNMTs, (ii) the prevalence of mutations and abnormal expression of DNMTs in cancer, (iii) factors that mediate their abnormal expression and (iv) the effect of anomalous DNMT-complexes in cancer.

GRAPHICAL ABSTRACT



DNA METHYLATION

DNA methylation involves the covalent addition of a methyl group (–CH3) to the 5′ position of cytosine (5mC) by DNA methyltransferases (DNMT) (1,2). The structures and catalytic mechanisms of DNA methyltransferases are highly conserved from bacteria to mammals; thus, DNA methylation is the most prevalent DNA modification (3). In mammals, it is predominantly found at CpG dinucleotides, 70–80% of which are methylated (4). DNA methylation is critical for mammalian development, differentiation, and its defects are implicated in several human diseases, including cancer (5).

In mammalian genomes, the non-uniform distribution of DNA methylation is strongly influenced by the overall depletion of CpG dinucleotides. Regions in mammalian genomes which contain expected or slightly higher levels of CpG are called CpG islands (CGI). DNA methylation

*To whom correspondence should be addressed. Tel: +1 765 494 3326; Email: hgowher@purdue.edu

shows the highest density at the repetitive and transposable elements and lowest levels at CGIs (6–8). CGIs are further categorized as high, intermediate, and low CG content. Interestingly, high CG content CGIs (>60%) are least methylated and are present at over two-thirds of all mammalian promoters, including housekeeping genes and a few developmental genes. The transcriptionally active state of housekeeping genes, which are critical for cellular function, is maintained by their unmethylated CGI promoters (9–11). Methylation changes associated with changes in gene expression are mostly found in intermediate and low CG content CGIs, which reside at tissue-specific promoters, differentially methylated regions (DMRs), enhancers, and super-enhancers (12–15). Interestingly, in cancer, enhancer DNA methylation was more closely associated with the aberrant gene expression profiles than the gene promoters, emphasizing the regulatory role of DNA methylation at low CG content sites (16–18). Regulation of CGI DNA methylation is also critical because aberrant CGI hypermethylation is prevalent in almost all cancers (19). Several mechanisms have been shown to regulate DNMT activity at CGI, including interactions with H3K4me, H3K36me and H4K20me3 histone tail modifications (20–23). Protection of CpGs from methylation can be achieved either directly by transcription factor (TF) binding or indirectly by TF-mediated targeting of chromatin remodeling enzymes (12,24,25). On the other hand, DNA methylation at regulatory elements can result in loss or gain of TF binding, affecting gene expression (26–28). In any case, aberrant methylation in cancer is either a direct consequence of DNMT mutations or indirectly due to misregulation of DNMTs, the enzymes that catalyze this modification.

Contrary to their regulatory elements, highly transcribed genes have abundant intragenic or gene body methylation (6). At intragenic regions, the predominant presence of the histone mark H3K36me3 recruits DNA methyltransferases (29,30). Correlation studies have demonstrated that gene body methylation antagonizes the activity of polycomb repressive complex (PRC2), hence, promoting transcription (31). Furthermore, DNA methylation plays a role in alternative promoter usage, alternative splicing, and in precluding cryptic transcription initiation (3,6,32–35). Aberrant hypermethylation of CGI's, which reside in the gene body, has been shown to correlate with increased expression of the corresponding genes and is predictive of elevated oncogene expression in cancer (36).

DNA methylation contributes to heterochromatin formation at repetitive and transposable elements by creating a target motif for methyl-binding domain (MBD) proteins (37,38). The histone modifiers such as methyltransferases and deacetylases in a complex with MBD proteins create a condensed chromatin state that prevents transcription (39,40). Aberrant hypomethylation and loss of heterochromatin are highly prevalent in cancer, leading to an increase in DNA recombination and loss of genome integrity. DNA methylation defects have been used as a sensitive marker for cancer diagnosis. Alone or in combination with other therapies, DNA demethylation therapy has been successfully used for some leukemias and myeloblastic syndromes (19). However, these methods are nonspecific and have global effects on DNA methylation, which can potentially have strong side effects and may even foster metastasis (41–44).

DNA METHYLTRANSFERASES

The DNA methyltransferases (DNMTs) are a family of enzymes that catalyze the transfer of the methyl moiety from the donor S-adenosylmethionine (SAM) to DNA (45). Mammalian DNMTs specialize in *de novo* methylation and maintenance methylation (46). The *de novo* methyltransferases (DNMT3A and DNMT3B) establish methylation patterns during embryonic development, while the maintenance methyltransferase DNMT1 copies methylation patterns from parent to daughter strand during DNA replication (47–49).

Structure/Function relationship of the DNMT1 methyltransferase

DNMT1 is a maintenance methyltransferase that is highly expressed in dividing cells (50). *DNMT1* knockout in mice results in a 90% loss of methylation and death mid-gestation (51). Inducible knockout of *DNMT1* in hESCs and human colon cancer cells HCT116 results in a rapid loss of methylation genome-wide accompanied by substantial cell death (52,53).

DNMT1 is a multi-modular enzyme comprising an N-terminus that contains a DNA-binding CXXC domain, a replication foci-targeting sequence (RFTS), two Bromo-adjacent homology (BAH) domains, and a C-terminal catalytic domain with an innate preference for hemimethylated DNA (54,55). The N-terminal domain of DNMT1 interacts with proteins that guide the methylation activity of DNMT1 (56). These interaction partners include PCNA (proliferating cell nuclear antigen), which targets DNMT1 to replication foci (57), the histone methyltransferase G9a (58) and HP1 (heterochromatin protein 1) (59). The DNMT1 RFTS domain, which constitutes the homodimer interface (60), interacts with the catalytic domain, preventing DNA binding (61,62). At hemimethylated sites, this autoinhibition is relieved by the interaction of the RFTS domain with UHRF1 (63). Additionally, the interaction of DNMT1 RFTS domain with H3K9me3 and H3Ub was recently shown to recruit DNMT1 to specific sites and increase its activity (64). The specificity of DNMT1 is further influenced by the interaction of its CXXC domain with unmethylated CpG sites. This interaction triggers a conformational change, positioning an autoinhibitory linker between the catalytic site and the DNA (65). Little is known about the role of the two tandem BAH domains, though they are conserved in all mammalian DNMT1 homologs (66). New insights, however show that the interaction of DNMT1 BAH domain with the repressive H4K20me3 is involved in heterochromatin formation (23).

The DNMT1 catalytic domain shares similarity in catalytic motifs with DNMT3 enzymes and is responsible for substrate binding, DNA binding, and catalysis (56). Though mammalian DNA methyltransferases utilize the same catalytic mechanism, they have unique catalytic parameters (46,55). DNMT1 is a highly processive enzyme and can methylate up to 30 CpG sites before dissociating

from the DNA, a well-adapted property for its role in maintenance methylation (67,68). Although DNMT1 has a high preference for hemimethylated DNA, it also performs *de novo* DNA methylation at unmethylated CCGG sites and targets transposable elements for *de novo* methylation during development (67,69–72). DNMT1 can also perform *de novo* methylation by cooperating with the DNMT3 *de novo* methyltransferases (73).

Structure/Function relationship of the DNMT3 methyltransferases

The DNMT3 family consists of two catalytically active members, DNMT3A and DNMT3B, and a catalytically-inactive member, DNMT3L (48,74). The DNMT3s are expressed primarily during embryonic development and in adult stem cells (75). DNMT3A and DNMT3B are highly homologous with roughly 40% sequence identity and a similar domain organization (76). *DNMT3B* knockout in mice is embryonic lethal, while *DNMT3A* knockout mice survive to term but die within six weeks after birth (47). In addition to unique targets, DNMT3 enzymes methylate many sites redundantly (52,77). DNMT3L is expressed explicitly during male and female germ cell development and plays an essential role in establishing methylation imprints (78–82). Knockout of *DNMT3L* in mice results in male sterility and defects in female oocytes (83–86).

The N-terminal regulatory regions of DNMT3A and DNMT3B consist of two domains, a Pro-Trp-Trp-Pro (PWWP) domain, and a cysteine-rich zinc-binding region called the ARTX-DNMT3-DNMT3L (ADD) domain (87). The PWWP domain interacts with both DNA and histone H3 methylated at the lysine 36 residue (H3K36me2/3), directing DNMT3 activity to intergenic regions (88–90). Studies show that DNMT3A interacts with H2AK119ub through an uncharacterized amino-terminal ubiquitin-dependent recruitment region targeting DNMT3A to specific regions (91). The ADD domain recruits DNMT3 enzymes to gene regulatory elements by binding to unmethylated histone H3 lysine 4 (H3K4) (92,93). When not bound to H3K4, the ADD domain interacts with the DNA binding region of DNMT3A, resulting in auto-inhibition (20,92).

The mammalian DNMTs contain ten conserved motifs in their C-terminal methyltransferase (MTase) domain. Motifs I – III are involved in SAM binding, while motifs IV and VI are required for catalysis (94,95). The target recognition domain (TRD), responsible for DNA binding, spans the region between the start of motif VII and the end of motif IX. The MTase domains of DNMT3A and DNMT3B share about 80% sequence similarity and can function independently of their N-terminal regions (96). DNMT3L lacks the motifs IX, X, and TRD, making it catalytically inactive (92). However, DNMT3L interacts with the catalytic domain of DNMT3A and DNMT3B, and allosterically stimulates their catalytic activity (80,84,97).

DNMT3A and DNMT3L co-crystallize as a hetero-tetrameric complex, with two DNMT3A monomers at the center, flanked by two DNMT3L monomers on either side (98). The two active sites of the central DNMT3A dimer are ∼40 Å apart, allowing for concurrent methylation of two CpGs separated by one helical turn of the DNA (87). In the absence of DNMT3L, DNMT3A can form homo-tetramers, which further oligomerize in the presence of DNA (32,99). The oligomerization of DNMT3A also supports its cooperative catalytic mechanism (99,100). The dimer interface of DNMT3A primarily constitutes the electrostatically interacting Arg and Asp residues (101). Given that DNMT3A monomers have low catalytic activity, the residues at the dimer interface are critical for optimal catalysis. This is further highlighted by a high prevalence of somatic substitution of the dimer interface residue, Arg882His, in acute myeloid leukemia (AML), resulting in about 80% loss of the catalytic activity of the mutant enzyme (102,103). Unlike DNMT3A, DNMT3B performs DNA methylation in a processive manner independent of interactions at the dimer interface (104). Although recent co-crystallization of DNMT3B with DNMT3L revealed a similar hetero-tetramer mediated by conserved residues, mutations of these residues in DNMT3B have little to no effect on the catalytic activity of the enzyme, emphasizing the specific role of dimer interface in the catalytic activity of DNMT3A (105). Recent structural analysis of the ternary complex of DNMT3A2, DNMT3B3 and a nucleosome core particle flanked by linker DNA indicates that the catalytically inactive accessory, DNMT3B3 binds to the acidic path of the nucleosome core, orienting the DNMT3A2 enzyme to bind to the linker DNA (106). These studies explain the observed methylation of linker DNA positioned nucleosomes by the DNMT3 enzymes (107).

CANCER-ASSOCIATED DNMT MUTATIONS

Recent large-scale cancer genomics consortia such as The Cancer Genome Atlas (TCGA) and the Genomics Evidence Neoplasia Information Exchange (GENIE) identified common somatic mutations across cancers. Whereas many epigenetic regulators carry somatic mutations, relatively few occur in the DNMT enzymes (reviewed in (108)). Mutations to DNMT1 have been identified in a small proportion of patients with colorectal carcinoma (109), while DNMT3B mutations have only been identified in patients with the genetic disorder immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome (110). Conversely, there is a high prevalence of DNMT3A somatic mutations in patients with acute myeloid leukemia (AML) (111). Some of these mutations have been extensively studied to understand their impact on DNMT catalytic activity. These data suggest a critical role of DNMTs in development, weak redundancy in their function, and lack of synonymity in their sequence.

DNMT3A mutations in acute myeloid leukemia

DNMT3A plays an important role in somatic stem cell differentiation in addition to its role in establishing methylation patterns during development (112). Knocking out DNMT3A in hematopoietic stem cells (HSCs) leads to decreased levels of cell differentiation and increased self-renewal (113). *DNMT3A−/−* HSCs display significant genome-wide hypomethylation with focal areas of hypermethylation (114,115). The phenotype of these cells is similar to what is observed in human hematological malignancies harboring DNMT3A loss-of-function mutations (116).

Genomic studies identified somatic DNMT3A mutations associated with poor prognosis in 22% of adult patients with acute myeloid leukemia (AML) (111,117). Similar DNMT3A mutations are also observed in chronic myelomonocytic leukemia (118). The PWWP and ADD domains harbor nonsense and frameshift mutations, while the MTase domain mostly acquires missense mutations (119). Most DNMT3A MTase domain mutations are located in the conserved motifs, dimer interface, and the TRD (119).

In AML, about 65% of DNMT3A heterozygous missense mutations affect codon Arg882, with the majority occurring as an Arg-to-His substitution (111,120). The mutation typically arises during the early stages of cancer development and is associated with significantly lower rates of overall and disease-free survival (121). This substitution disrupts intermolecular interactions at the dimer interface and decreases DNA binding, resulting in a 40–80% loss of catalytic activity (99,101,103,122). Additionally, the DNMT3A Arg882His variant has altered flanking sequence preference around the target CpG site compared to wild-type DNMT3A (123). The flanking sequence preference of the DNMT3A Arg882His variant resembles DNMT3B more closely than that of WT DNMT3A, which may lead to aberrant methylation of DNMT3B targets in AML (124) (Figure 1A). In addition to altered flanking sequence preference, the Arg882His variant has weak interface interactions that disrupt the cooperative mechanism (124) (Figure 1B). Through its interaction with the WT enzyme, the Arg882His variant is suggested to have a dominant-negative effect on cooperativity (103,122,125). Other variants of Arg882, such as Arg882Cys, Arg882Ser and Arg882Pro are also found in AML patients and were shown to have reduced catalytic activity similar to Arg882His, with some differences in catalytic properties (126).

Aberrant expression of MEIS1 has been previously associated with poor prognosis in AML (127), and DNMT3A mutation can activate the MEIS1-mediated transcription program following *MEIS1* promoter hypomethylation (128). *In vitro* studies using MEIS1 enhancer as a substrate showed significantly lower activity of DNMT3A Arg882His variant at all except one CpG site with flanking sequence preferred by DNMT3B, emphasizing the influence of this AML mutation on the substrate specificity of the enzyme (124). These *in vitro* experiments suggest that besides causing genome-wide hypomethylation due to loss of catalytic activity, the AML mutation can result in a gain of function activity by which the variant DNMT3A enzyme could preferentially methylate DNMT3B target sites. Although biological outcomes of a change in the substrate preference have not been described, the data suggest a potential occurrence of aberrant methylation leading to changes in gene expression. Hypomethylation resulting from loss of DNMT3A catalytic activity causes widespread gene dysregulation, including the overexpression of the *Hox* family genes and *Idh1* (129). Conditional expression of the mouse DNMT3A R878H mutant (the mouse equivalent of R882H) initiates AML and mimics features of human leukemia (130). The mechanism of pathogenesis in this mouse model was found to be related to aberrant *mTOR* activation resulting from DNA hypomethylation (130).

Over ten additional missense DNMT3A mutations have been identified in AML patients, but they occur at lower frequencies than the Arg882His. *In vitro* studies of DNMT3A variants such as Arg635Gly, Ser714Cys, Trp893Ser, Pro904Leu, Arg736His and Arg771Gln/Pro/Gly displayed reduced catalytic stimulation by DNMT3L and a substrate-dependent decrease in catalytic activity, suggesting biological effects similar to Arg882His variant (131).

DNMT GENE EXPRESSION CHANGES IN CANCER

Alterations in DNA methylation patterns can result in changes in oncogene and tumor suppressor gene expression (132). DNA methylation is maintained through a myriad of factors, including the DNA methyltransferases, TET methylcytosine dioxygenases and histone-modifying enzymes. As the proteins chiefly responsible for establishing and maintaining methylation patterns, the DNMTs have been widely implicated in methylation changes in cancer cells. Significant focus has been placed on studying the consequences of DNMT expression changes observed in various cancers.

Mouse models of cancer have proven extremely useful in ascertaining the role of DNMTs in cancer pathogenesis. In a mouse model of pancreatic cancer, DNMT1 hypomorphic mice had a reduction in tumor burden coupled with decreased DNA methylation at a subset of cancer-associated genes in the pancreas (133). These findings are relevant to the observation that aberrant DNA hypermethylation is observed in tumors from early- and late-stage pancreatic cancer [120, 121], indicating that DNMT1 may drive some of these initial altered methylation patterns. In a mouse model of intestinal neoplasia, overexpression of DNMT3B1 resulted in enhanced colon tumorigenesis and tumor size (134). DNMT3A inhibition in a mouse model of melanoma inhibited tumor growth and affected the expression of various tumor-related genes, including class I and II MHC genes and various chemokines (135). These studies cumulatively imply a role for DNMTs in enhancing the pathologic characteristics of cancer cells.

Some studies also point to DNMTs as having roles as tumor suppressors in cancer. In mouse models of lung adenocarcinoma, DNMT3A knockout negatively impacts angiogenesis and cell adhesion (136). This observation is in line with a study showing that increased DNMT3A expression in lung adenocarcinoma is associated with a favorable prognosis (137). Similarly, DNMT3A deficiency results in attenuated progression of peripheral T cell lymphoma (138), and DNMT3B loss hastens the progression of MLL-AF9 leukemia (139). These studies exemplify that DNMT expression level changes can have broad effects that lead to pathogenesis.

Effect on the global methylation pattern

Altered genome-wide methylation has long been related to genome instability, increased chromosomal translocations, and widespread gene dysregulation in cancers (132,140–142). Therefore, overall changes in methylation related to DNMT expression variation are critical in cancer pathogenesis.

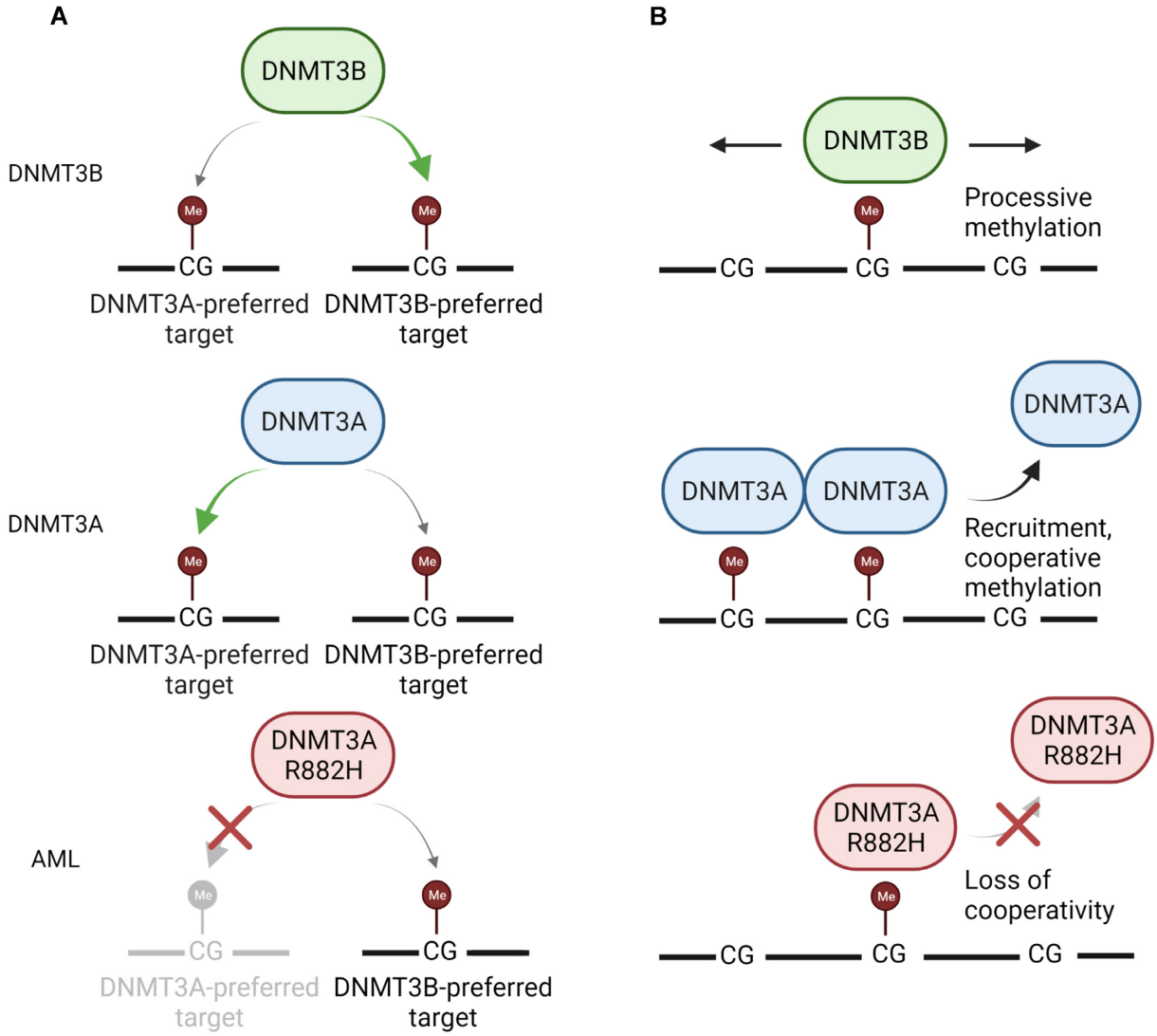


Figure 1. Catalytic properties of DNMT3 enzymes and the DNMT3A R882H mutant. (**A**) DNMT3A and DNMT3B preferentially methylate specific targets. The DNMT3A R882H is a somatic mutation found predominantly in AML patients, accounting for about 65% of all DNMT3A mutations in AML, and at lower frequencies in other cancers such as MDS, MPN, T-ALL and AITL (116,268). The DNMT3A R882H mutation ablates the ability of DNMT3A to target DNMT3A-preferred sites, while its activity at DNMT3B-preferred sites is unaffected. (**B**) DNMT3B methylates CpG sites in a processive manner, whereas DNMT3A methylates cooperatively by recruiting additional DNMT3A subunits. The DNMT3A R882H mutation also disrupts DNMT3A cooperativity that reduces the overall catalytic activity of the enzyme. AITL: angioimmunoblastic T- cell lymphoma; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasms; T-ALL: T-cell acute lymphoblastic leukemia.

Publicly available data from The Cancer Genome Atlas (TCGA) illustrates that significant alterations in DNMT mRNA expression are observed in a wide variety of cancers (Figure 2). Although the gene expression data in Figure 2 are not normalized to proliferation-specific control (143), the overexpression of DNMTs in most of these cancers has been verified in several studies (reviewed in (144,145)). High DNMT3B expression levels correlate with a particular colorectal cancer phenotype characterized by high global DNA methylation levels, particularly at CpG islands (146). In colorectal and gastric cancers, overexpression of DNMT3B was also correlated with disease progression and increased levels of methylation in tumor cells (147,148). Irrespective of changes in DNMT expression, genome-wide hypomethylation coupled with focal hypermethylation is a feature of almost all cancers (reviewed in (149)). Hypomethylation itself has been tied to the pathogenicity of various cancers. For example, increased chromosomal rearrangement is associated with DNA hypomethylation in hepatocellular carcinoma (150) and prostate cancer (151). Dnmt1 knockout mice show genomic instability, hypomethylation of repetitive elements, and increased macroadenoma load in intestinal cancer (152).

Tumor suppressor gene silencing

TSG silencing resulting in cancer pathogenesis is a pervasive feature, making the mechanisms resulting in this silencing an intriguing avenue for cancer research. In many cases,

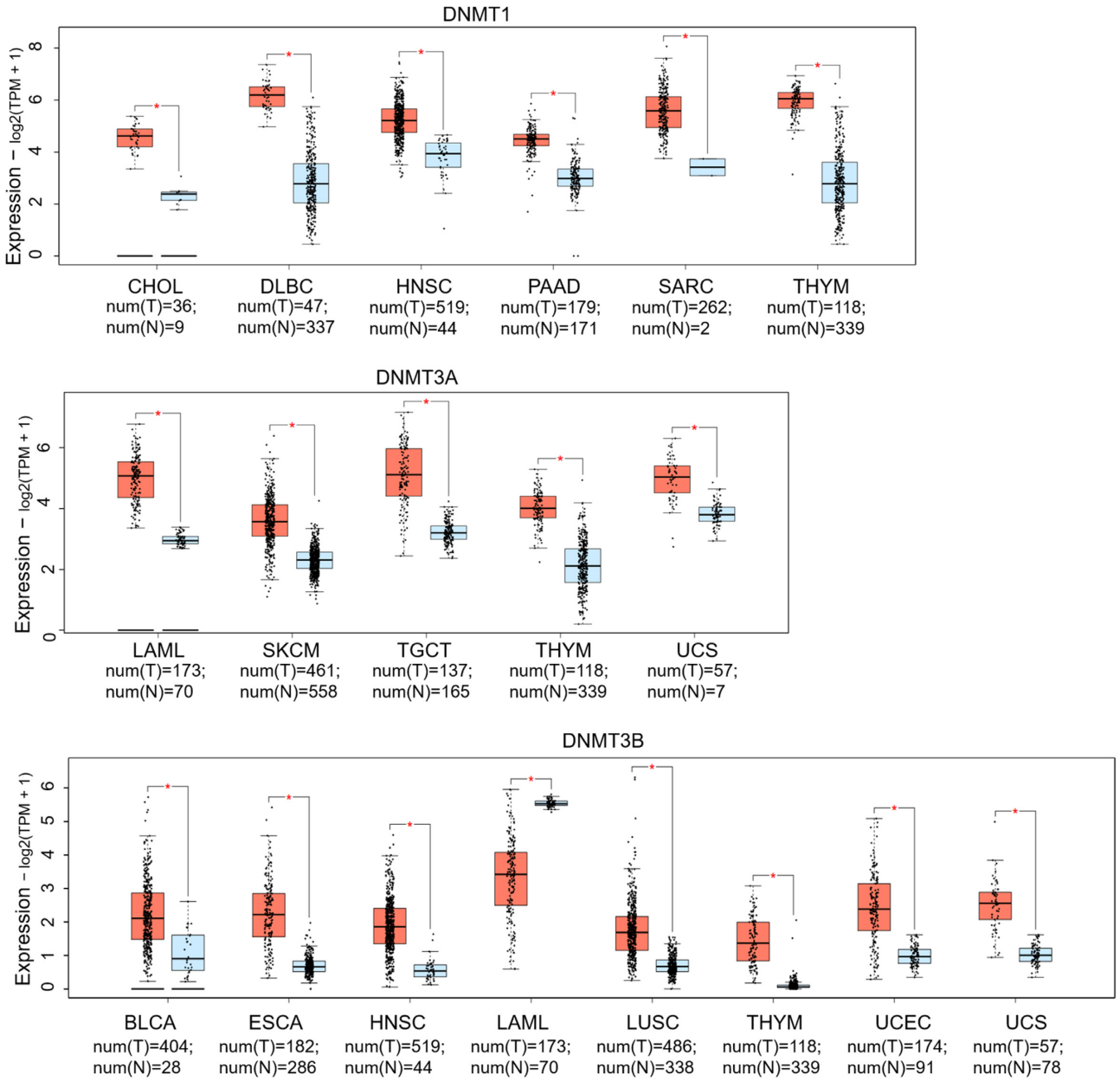


Figure 2. Differential expression of *DNMTs* from TCGA data. Box plots of RNA-seq data comparing TCGA tumor samples (red) to TCGA normal and GTEx samples (blue). *P*-value cutoff = 0.01. Box plots were generated using GEPIA 2 (269). BLCA – bladder urothelial carcinoma, CHOL – cholangio carcinoma, DLBC – lymphoid neoplasm diffuse large B-cell lymphoma, ESCA – esophageal carcinoma, HNSC – head and neck squamous cell carcinoma, LAML – acute myeloid leukemia, LUSC – lung squamous cell carcinoma, PAAD – pancreatic adenocarcinoma, SARC – sarcoma, SKCM – skin cutaneous melanoma, TGCT – testicular germ cell tumors, THYM – thymoma, UCEC – uterine corpus endometrial carcinoma, UCS – uterine carcinosarcoma.

the altered expression of a DNMT can result in aberrant methylation at TSG promoters, resulting in TSG silencing in cancer cells.

DNMT3A overexpression in gastric cancer leads to silencing the *p18INK4C* gene, a cyclin-dependent kinase inhibitor that regulates cell cycle progression (153). The silencing of *p18INK4C* by DNMT3A leads to cell cycle dysregulation and accelerated G1/S transition, promoting cancer progression (153). In hepatocellular carcinoma (HCC), 64% of tissue samples overexpressed DNMT3A, and it was implicated in the methylation and subsequent silencing of the PTEN gene, which encodes a phosphatase critical for cell cycle regulation (154). In lung squamous cell carcinoma, DNMT1, DNMT3A and DNMT3B are expressed at higher levels in cancer tissue relative to healthy tissue controls (155). DNMT1 and DNMT3B expression in cancerous lung tissue is correlated with gene promoter hypermethylation at multiple TSG promoters, including *FHIT* and $p16^{INK4a}$ (155). A recent study found that in endometrial cancer, concurrent DNMT3B and EZH2 upregulation in cancer cells results in the epigenetic silencing of TCF3 (156). TCF3 is a transcriptional activator of CCKN1A

($p21^{WAF1/Cip1}$), and its silencing results in accelerated endometrial cancer cell proliferation (156).

Gene body methylation of oncogenes

Though DNA methylation at gene promoters is associated with transcriptional silencing, methylation in gene bodies is associated with the active transcription of genes (36). Gene body methylation of oncogenes, therefore, can result in oncogene overexpression, driving cancer pathogenesis. A broad analysis of seven solid tumor types found a correlation between gene body hypomethylation and overexpression of ∼43% of homeobox genes, many of which are oncogenes (157). Researchers found that this overexpression could be recapitulated by targeting DNMT3A to specific homeobox gene bodies for hypermethylation (157). In liver cancer, gene body and 5′-UTR methylation of oncogenes are associated with their overexpression (158). This pattern of oncogene gene body methylation and increased gene expression was observed in 56% of patients (158). Treatment of HCC cells with the DNMT inhibitor decitabine reduced gene body methylation, gene expression levels and transiently reduced the tumorigenic properties (158). In renal clear cell carcinoma and lung adenocarcinoma, the *CARD11* gene is significantly overexpressed concurrent with increased *CARD11* gene body methylation relative to healthy cells (159). In the context of these cancers, CARD11 aberrantly activates the mTOR pathway and suppresses autophagy (159). Again, treatment of cells from both cancer types with decitabine decreased *CARD11* expression (159), suggesting a direct role DNMTs in hypermethylation and cancer-specific changes in gene expression.

Cancer cell growth and proliferation

Aside from DNA methylation and gene expression changes, DNMT expression level alterations have been linked to various other oncogenic properties in cancer cells, including cancer stem cell maintenance and proliferation.

The ability of colon cancer cells to initiate tumors was significantly reduced upon DNMT1 knockout, but their ability to grow otherwise was not affected (160). This observation is partially attributed to a reduced proportion of cancer-initiating cells in the DNMT1 knockout colon cancer cell line (160). DNMT1 was also found to be necessary for the maintenance of bilinear myeloid-B lymphoid leukemia stem cells (161). In mammary cancer, DNMT1 loss results in limitation of the cancer stem cell population (162). These studies indicate a potentially larger role for DNMT1 in cancer stem cell maintenance, reminiscent of DNMT's critical role in maintaining several human stem cell lineages (52,162,163).

Other than stem cell maintenance, DNMTs have also been found to affect cancer cell pathogenic properties, including proliferation and migration. In Burkitt lymphoma, both DNMT1 and DNMT3B are overexpressed, and treatment of Burkitt lymphoma cell lines with the DNMT inhibitor decitabine decreases DNMT levels and inhibits cell growth (164). DNMT3L expression supports the growth of embryonal carcinoma cells and is known to be significantly overexpressed in testicular germ cell tumors (165).

Prognostic value

Given its high prevalence in various cancers, DNMT expression level changes can predict patient prognosis and stage cancers. For example, in glioblastoma, DNMT3B and DNMT1 are highly overexpressed, and their expression levels can be used as markers for cancer staging (166). Investigation into these expression changes revealed hypomethylation at the DNMT1 and DNMT3B gene promoters along with a distinct euchromatin signature at the DNMT1 promoter in tumors (167). Similarly, in AML, DNMT3B expression at high levels independently carries an unfavorable patient prognosis (168), in addition to the known poor prognosis for patients carrying mutations in DNMT3A discussed above (169,170). Pancreatic ductal adenocarcinoma (PDAC) patients with higher levels of DNMT1 have a lower survival rate than those with lower expression (171). In PDAC, high DNMT1 levels were also correlated with nerve infiltration, TNM staging, degree of cell differentiation, and advanced stages of the disease (171). In chronic pancreatitis, DNMT3A and DNMT3B expression is correlated with tumor size, and patients with higher DNMT3A and DNMT3B expression have a lower survival rate (172). DNMT3B is also overexpressed in endometrial cancer and is even more highly expressed in poorly-differentiated than well-differentiated endometrial cancer cell lines (173). In agreement with the role of DNMT3B in endometrial cancer pathogenesis, treatment of an endometrial cancer cell line with a DNMT inhibitor inhibited cell proliferation and increased apoptosis (174). Cumulatively, research points toward DNMT levels being a physiologically relevant readout of cancer progression and attractive as a means of deducing patient prognosis.

MECHANISMS CONTROLLING DNMT EXPRESSION IN CANCER

Since changes in DNMT expression levels can have severe consequences in terms of cancer patient prognosis, the mechanisms by which DNMT expression is dysregulated in cancer have also been thoroughly investigated. DNMT expression can be altered through many mechanisms, including aberrant *DNMT* pre-mRNA splicing, polymorphisms within *DNMT* promoters, and epigenetic mechanisms at *DNMT* gene regulatory elements.

Transcription factor complexes

Alterations in DNMT expression levels are often attributed to aberrantly expressed transcription factor complexes or epigenetic remodelers acting upon the *DNMT* promoter regions. In non-small cell lung cancer, overexpression of DNMT1 along with the transcription factor Sp1 is associated with poor patient prognosis (175). The overexpression of DNMT1 was found to be partly mediated by *p53* mutations that abrogate its interaction with Sp1 at the *DNMT1* promoter, which would normally repress *DNMT1* expression (175). DNMT3A and DNMT1 are both overexpressed in pancreatic cancer, in which GLI1 promotes the expression of both DNMT3A and DNMT1 (176). GLI1 is an effector protein in the Hedgehog signaling pathway, which is aberrantly activated in pancreatic cancer (177). In lung

adenocarcinoma, the HOXB3 transcription factor binds the *DNMT3B* gene and increases DNMT3B expression (178). This increased DNMT3B expression leads to the epigenetic silencing of the *RASSF1A* tumor suppressor gene, which could be reversed using DNMT inhibitor treatment (178). In breast cancer, *DNMT3A* expression is repressed by the MTA1/HDAC1/YY1 co-repressor complex (179). High expression of MTA1 coupled with low DNMT3A expression predicts a poor prognosis for breast cancer patients (179).

Viral proteins

Multiple cancer-associated viral proteins have been implicated in DNMT expression alterations, leading to genome-wide methylation pattern changes and gene dysregulation (Reviewed in (180)). In hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC), the overexpression of the viral-encoded HBx regulator protein can upregulate DNMT1, DNMT3A1 and DNMT3A2 (181). The upregulation of these DNMTs is coincident with the overall hypomethylation coupled with focal hypermethylation phenotype observed in HCC tumor cells (182). The hepatitis delta virus (HDV) enhances the development of hepatocellular carcinoma in infected patients (183). The HDV-encoded delta antigen upregulates DNMT3B by activating the STAT3 pathway, resulting in hypermethylation at specific loci, including the TSG transcription factor *E2F1* (184). Hepatitis C virus (HCV) -positive HCCs have a unique DNA methylation signature consisting of both DNA hypo- and hypermethylation (185,186). Consistently, the HCV core protein was shown to upregulate both DNMT1 and DNMT3B, resulting in promoter hypermethylation of the *CDH1* TSG (187). These studies indicate that DNMT overexpression downstream of hepatitis viral proteins is an overarching pathogenic mechanism in hepatitis-associated HCC.

Promoter polymorphisms

Polymorphisms in the DNMT promoter regions that alter promoter activity have been characterized in multiple cancers. The DNMT3B promoter −149C→T single-base transition, correlated with increased promoter activity, is associated with more than a two-fold increased risk of developing lung cancer (188). This same promoter polymorphism is also associated with an earlier onset of hereditary nonpolyposis colorectal cancer in patients with this polymorphism than those carrying the wild-type allele (189). The DNMT3B promoter −579G→T polymorphism is associated with a higher risk of developing thymoma in myasthenia gravis patients (190), along with a higher risk of developing multiple other cancers, including lung cancer and head and neck squamous cell carcinoma (191,192). The DNMT3A promoter −448A→G polymorphism is a common SNP associated with alterations in DNMT3A promoter activity (193). The −448A allele increases promoter activity and has been associated with an increased risk of developing gastric cancer (193).

Alternative splicing

Aberrant alternative splicing can also be a component of altered DNMT expression in cancer, especially in the case of DNMT3B, which has over 30 described splice isoforms resulting from alternative splicing events and alternative promoter usage (194,195). In Myc-induced lymphoma, expedited lymphomagenesis is associated with increased expression of a truncated catalytically-inactive dominant-negative isoform, DNMT3B7 (196). This observation is corroborated by a recent study that showed that mice expressing a catalytically-inactive DNMT3B isoform from one or both alleles develop B-cell lymphomas among other hematologic malignancies (197). In non-small cell lung cancer (NSCLC), the *ΔDNMT3B* subfamily was described, consisting of at least seven DNMT3B variants resulting from alternative splicing, some lacking enzymatic activity (198). These different *ΔDNMT3B* isoforms can differentially regulate methylation of specific genes, including the tumor suppressor *RASSF1A*, which is specifically methylated by the *ΔDNMT3B* family member *ΔDNMT3B4* (199). A recent study found that DNMT3B isoforms lacking catalytic activity were highly expressed in HCC relative to healthy liver tissue (200). Upon hepatocyte-specific *DNMT3B* deletion, mice exhibit a higher incidence of HCC relative to control mice, suggesting that catalytically-active 3B may play a protective role against hepatocarcinogenesis (200).

INTERACTIONS OF DNMTS AND THEIR MISREGULATION IN CANCERS

The interactions of DNMTs with proteins and post-translationally modified histones regulate DNA methylation at specific genomic regions in various cell types (6,97,201,202). Spurious DNA methylation in cancer may be caused by the disruption of conventional DNMT interacting partners or the formation of new complexes due to the aberrant expression of developmental factors that misdirect DNMTs to atypical genomic sites (Table 1).

Interaction of DNMTs with DNA binding factors

Site-specific DNA methylation is regulated by transcription factor-mediated recruitment of DNMTs to their binding sites, leading to specific changes in gene expression (Figure 3). For example, p53, a tumor-suppressing transcription factor, interacts and stimulates DNMT1 activity, which consequently represses the SURVININ promoter in human fibroblasts (203). In osteosarcoma cells, MYC targets DNMT3A to MYC-specific gene promoters to mediate gene repression (204). In NIH3T3 cells, PU.1, a master regulator for myeloid and B-cell lineage development, recruits DNMT3A/B to methylate promoters of the TSGs *p16* and *p27* (205). Further, in HCT116 cells, DNMT3A interacts with DAXX and functions as a co-repressor for DAXX target genes independent of its methyltransferase activity (206). PML-RARα, a chimeric oncoprotein formed by an aberrant translocation of PML gene next to RARα, recruits DNMT3A and DNMT1 to silence the promoter of the tumor suppressor RARβ2 gene in acute promyelocytic leukemia (207). DNMT3A also interacts with ISGF3γ, a

Table 1. List of DNMT interacting partners in human cancers. This table shows a list of DNMT interactions with proteins which play important roles in several human cancers as described in the listed references

	Interacting partner (s)	DNMT	Evidence in human cancer	Ref.
DNA-bound proteins	CFP1	DNMT1	Disruption of this interaction reduces tumor growth in glioma cells.	(214)
	p53	DNMT3A	Represses p53-mediated gene expression	(221)
	DAXX	DNMT1	DAXX recruits DNMT1 to specific genomic loci to regulate autophagy programs in prostate cancer	(270)
	MECP2	DNMT1	Interaction mediates hypermethylation at *ESR1*, *survivin* and *cdc25c* genes in breast cancer.	(203)
	PCNA	DNMT1	Disruption of this interaction is an oncogenic event in tumorigenesis	(220)
	MYC	DNMT3A	Induces promoter methylation and miR-200b silencing in breast cancer	(271)
	Rb	DNMT1	Rb inhibits the methyltransferase activity of DNMT1, which may lead to global hypomethylation in osteosarcoma	(212)
	MAFG	DNMT3B	Silencing of CIMP genes in colorectal cancer	(209)
	ZNF304	DNMT1	Silencing of CIMP genes in colorectal cancer	(210)
	PU.1	DNMT3A/B	Recruits DNMTs to silence TSGs in NIH3T3 cells	(205)
	PML-RARα	DNMT3A/ DNMT1	Silences the RARβ2 gene in acute promyelocytic leukemia	(207)
	ISGF3γ	DNMT3A	Disruption of this complex enhances the efficiency of chemotherapy in mice tumors	(208)
	UHRF1	DNMT1	Disruption of this complex induces tumorigenesis in astrocytes, breast, lung, and mesothelial cells	(219)
	STAT3	DNMT1	May be involved in STAT3 mediated transcriptional repression of tumor suppressor genes	(272)
Chromatin-modifiers	SETDB1	DNMT3A	Mediates transcriptional repression of tumor suppressor genes	(231)
	hNaa10p	DNMT1	Recruits DNMT1 to suppress TSG expression and enhances DNMT1 activity	(273)
	HDAC1/2	DNMT1/DNMT3A/B	Mediates maintenance of heterochromatin in normal and cancer cells	(211,233,274)
	HP1	DNMT1	Disruption of this interaction promotes tumorigenesis in mice	(227)
	LSH	DNMT3B	Depletion of LSH reduces DNMT3Bs' association with DNA in erythroleukemia	(245)
	MBD3	DNMT3A/B	Mediates gene silencing on the MT-1 promoter in lymphosarcoma cells	(241)
	NsPc1	DNMT1	Silences HOX genes	(232)
	DMAP1	DNMT1	Disruption of this interaction increased sensitivity of cancer cells to chemotherapy in colorectal cancer	(227,234)
	USP7	DNMT1	Interacts with and stabilizes DNMT1, promoting the catalytic activity of DNMT1	(275,276)
	EZH2	DNMT1	Mediates silencing of miR-484 and contributes to cervical cancer progression	(277)
	KDM1A	DNMT1	Localized to heterochromatin in a cell cycle-dependent manner in cancer cells	(237)
	G9a	DNMT1	Coordinates DNA and histone methylation during replication and has been implicated in small cell lung cancer	(278,279)
	PRC1	DNMT3A R882H	Silences differentiation in a DNA-methylation independent manner	(230)
	Suv39H1	DNMT1/3A/3B	May be responsible for Snail-mediated E-cadherin repression in breast cancer	(280)

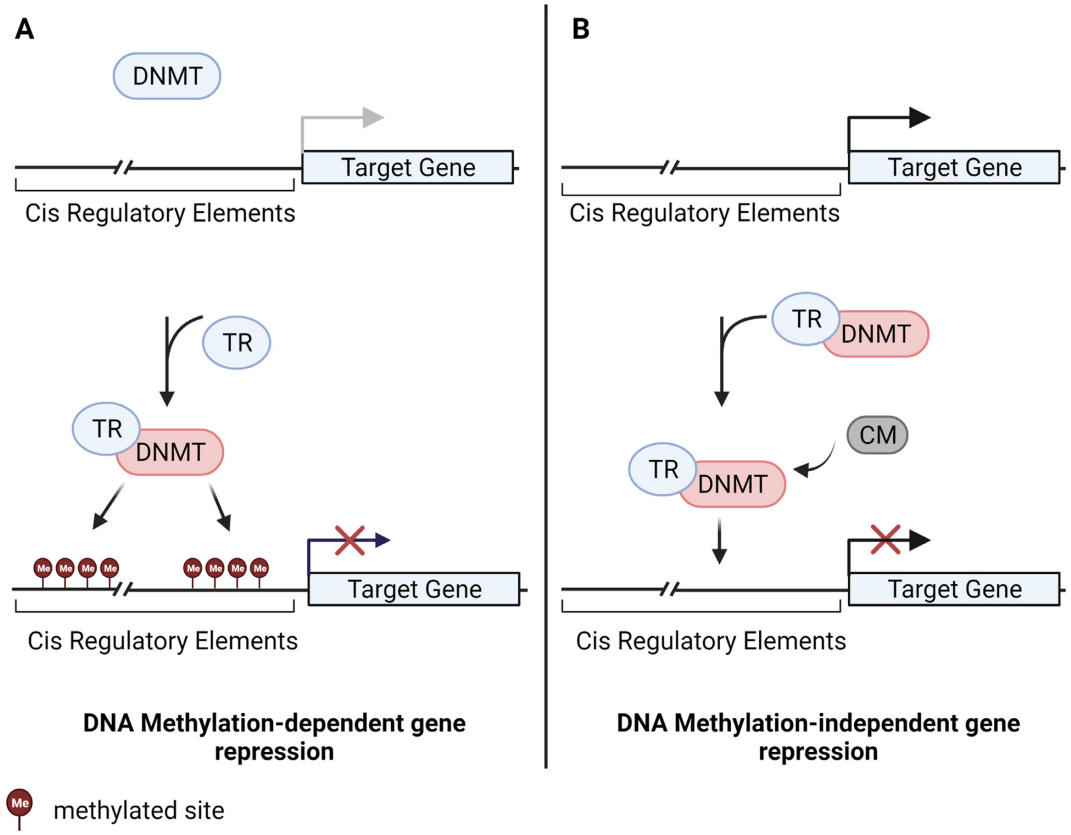


Figure 3. Schematic representation of transcription factor-mediated recruitment of DNMTs in cancer. (**A**) Unmethylated promoter regions typically promote transcription. Transcriptional repressors potently recruit DNMTs to mediate site-specific DNA methylation at promoter or enhancer regions that attenuate transcription of genes, especially TSGs in cancer. (**B**) Gene repression by recruitment of DNMTs by transcription factors can be independent of the methyltransferase activity of DNMTs. TR-bound DNMTs may function as an artificial co-repressor or recruit chromatin-modifiers that mediate gene repression at target sites. DNMT – DNA methyltransferase; -Me – methyl group on methylated DNA; CM – chromatin modifier. TR – Transcriptional repressor .

transcription activator induced upon interferon α stimulation (202). High levels of DNMT3A/ISGF3γ complex indicate a poor prognosis in tumors, and subsequent disruption of this interaction enhances the efficiency of chemotherapy in mice tumors (208). It is proposed that MAFG, a transcriptional repressor, recruits a co-repressor complex that includes DNMT3B to methylate and silence CpG island methylator phenotype (CIMP) genes in BRAF mutant colorectal tumors (209). Similarly, promoter-bound ZNF304 recruits DNMT1 as part of a co-repressor complex to methylate and silence transcription of CIMP genes in KRAS mutant colorectal cancer (210).

DNMT1, via its CXXC domain, directly interacts with the Rb TSG and represses reporter constructs containing E2F binding sites without detectable changes in promoter methylation (211). On the other hand, in osteosarcoma cells, the interaction of Rb with DNMT1 was shown to inhibit its methyltransferase activity by disrupting DNA/DNMT1 complexes, contributing to global hypomethylation defects, which is a general phenomenon in most cancers (212). CXXC finger protein 1 (CFP1), a component of the Setd1A and Setd1B methyltransferase complexes, directly interacts with DNMT1. CFP1-deficient ESCs show global hypomethylation and loss of DNMT1 protein suggesting the role of CFP1 in DNMT1 protein stability. (213). Disruption of the DNMT1/CFP1 interaction strongly increases the sensitivity of tumors to chemotherapy in mice and reduces tumor growth of glioma cells (214). Based on more recent studies demonstrating a robust allosteric regulation of DNMT1 activity (215), it will be interesting to study the impact of the Rb/DNMT1 and CFP1/DNMT1 interaction on the activity and specificity of DNMT1 in normal and cancer cells.

DNMT1 also interacts with PCNA, a processivity factor of DNA polymerase, to mediate post replication maintenance of DNA methylation (216,217). p21, a cell cycle regulator, and DNMT1 interact with PCNA in a mutually exclusive manner (216). It is speculated that the loss of p21 in most cancers may result in an opportunistic interaction of PCNA with DNMT1 leading to mistargeting and spurious DNA methylation (218). However, the disruption of DNMT1/PCNA interactions promotes carcinogenesis and tumorigenesis in several human cancer cells (219,220). Thus, it is imperative to study the mechanisms underlying the Ying Yang effects caused by DNMT interactions in cancer. Additionally, DNMT3A directly interacts with p53 to repress p53-mediated transactivation of p21 in breast cancer cells, independent of its methyltransferase activity (221). Altogether, with or without affecting their methyltransferase activity, the interactions of DNMTs with other DNA bind-

ing complexes are essential for the proper regulation of gene expression and genome stability, misregulations of which are implicated in cancer.

Interaction of DNMTs with chromatin modifiers

Anomalous interactions between chromatin-modifying proteins with DNMTs have been implicated in cancer. Underscoring a functional relationship that facilitates the cooccurrence of deacetylated histones and DNA methylation, early studies showed interactions between histone deacetylases HDAC1 and HDAC2 with DNMT3A/B and DNMT1 in mammalian cells (222–224). In human cancer cells, the combined activity of these enzymes has been targeted by a combination of inhibitors which leads to the re-expression of densely hypermethylated and transcriptionally silenced TSGs. (225). Loss of DNMT1 in HCT116 cells is associated with an increase in H3K9 acetylation and a decrease in H3K9 methylation with concomitant loss of HDACs and HP1, suggesting that DNMT1 mediates the maintenance of heterochromatin in human colon cancer cells (226). Additionally, the disruption of DNMT1 interaction with DNMT3B or HP1 promoted tumorigenesis in mice (227). Repressive chromatin is also induced by an interaction of DNMTs with the SUV39H1 and EZH2 histone methyltransferases, which methylate histone H3K27 (46,59,228). Studies using peptides to disrupt DNMT3B interaction with HDAC1 and EZH2 enhanced tumorigenesis in a mouse glioma model (227). Although DNMT3A interacts with polycomb group proteins to mediate gene repression through its DNA methylation activity (229) the DNMT3A R882H AML variant interacts and recruits PRC1 to the PU.1 URE region, suggesting a DNA methylation-independent silencing of cell differentiation and lineage commitment in leukemogenesis (230).

Other examples of a collaboration between DNMTs and chromatin-modifying enzymes include the specific interaction of DNMT3A with the histone methyltransferase, SETDB1, that localizes DNMT3A to methylate and inactivate the promoter of RASSF1A, a TSG commonly silenced in human cancers (231). DNMT1 associates with a neural-specific polycomb, NSPc1 and EzH2 to form a complex that silences *HOX* genes (232). Some interactions were shown to affect the DNMT activity, including DMAP1−DNMT1 interaction, which enhanced the enzymatic activity of DNMT1 (233,234). DMAP1 participates in the TIP60-p400 histone acetyltransferase (HAT) complex, which acetylates histone H4 at lysine 16 (H4K16) to relax condensed chromatin (235). Disrupting DMAP1-DNMT1 interaction resulted in an increased sensitivity of glioma cancer cells to chemotherapy and irradiation-induced cell death potentially due to repression of TSGs (227). Correspondingly, the reduction in DMAP1 protein by lentiviral shRNA showed a decrease in DNA methylation at the p16 promoter with a concomitant reduction in cell proliferation (234). Additionally, DNMT1 activity is affected by its interaction with LSD1. Demethylation of DNMT1 protein by LSD1 was shown to be essential for its stability. Although the loss of LSD1 in embryonic stem cells resulted in progressive loss of DNA methylation (236), depletion of LSD1 in cancer cells had no such effect (237). In cancer cells, however, LSD1–DNMT1 interaction is highest during the S-phase, suggesting a role of this interaction in cell cycle progression and pathogenesis (237–239).

At repetitive elements, DNMT3A and DNMT3B interact with specific histone modifications and the heterochromatin binding protein 1 (HP1), thus accumulating DNA methylation at these genomic elements (47,88,240). DNMTs also interact with MBD2/3 and MeCP2 methyl CpG binding proteins, leading to gene repression in normal and cancer cells (46). For instance, in mouse lymphosarcoma cells, DNMT3A/B interacts with MBD3 to mediate gene silencing on the MT-I promoter (241). An ATP-dependent chromatin remodeling protein, LSH, associates with DNMTs to mediate DNA methylation at specific genome sites and repress transcription (242,243). Consistent with the observation that DNA hypomethylation is prevalent in cancer (244), the deletion of LSH in mice resulted in the reduced association of DNMT3B with DNA and the global loss of DNA methylation leading to the development of erythroleukemia (245).

Interaction of DNMTs with modified histone tails

DNMTs interact with specific post-translational modifications on histones through their N terminal motifs, thus potentially targeting DNA methylation and regulating the regional specificity of DNMTs (reviewed in (246)). DNMT3 enzymes have two chromatin interacting domains, the ADD (ATRX–DNMT3–DNMT3L) domain, and the PWWP domain, both of which mediate interactions of DNMTs with chromatin and regulate their activity on nucleosomal DNA. (247). The PWWP domain of DNMT3B interacts with histone H3K36me3, and its catalytic domain allosterically interacts with DNMT3A catalytic domain. Thus a concerted interaction of DNMT3B3, a catalytically inactive isoform of DNMT3B, with DNMT3A and histone H3K36 was shown to recruit and enhance the activity of DNMT3A at H3K36me3 gene regions and CpG islands in colorectal cancer cells (248,249). Similarly, DNMT3A via its PWWP domain interacts with H3K36me2 to mediate intergenic DNA methylation (22). The ADD domains of DNMT3L and DNMT3A specifically interact with unmethylated H3K4, thereby triggering de novo DNA methylation at these sites (92,93,250). An interaction between the ADD domain of DNMT3A with H3K4me0 releases DNMT3A from an autoinhibited conformation (20,92,93,251) (Figure 4). These observations are consistent with findings that DNA methylation inversely correlates to H3K4 methylation in embryonic stem and somatic cells (11,252,253). This mechanistic feature allows dynamic regulation of DNMT3A activity wherein loss of histone H3K4me can potentially recruit/trigger the activity of DNMT3A via the interaction of its ADD domain with unmethylated histone H3K4. Indeed recent studies elucidated such a mechanism that involves the interaction of DNMT3A with the LSD1–Mi2/NuRD complex and targets DNA methylation to the enhancers of pluripotency genes during ESC differentiation. As pluripotency is turned off post differentiation, repression of PpGs is orchestrated by a series of chromatin-associated enzymatic activities of the LSD1–Mi2/NuRD – DNMT3A complex. Histone deacetylation and demethyla-

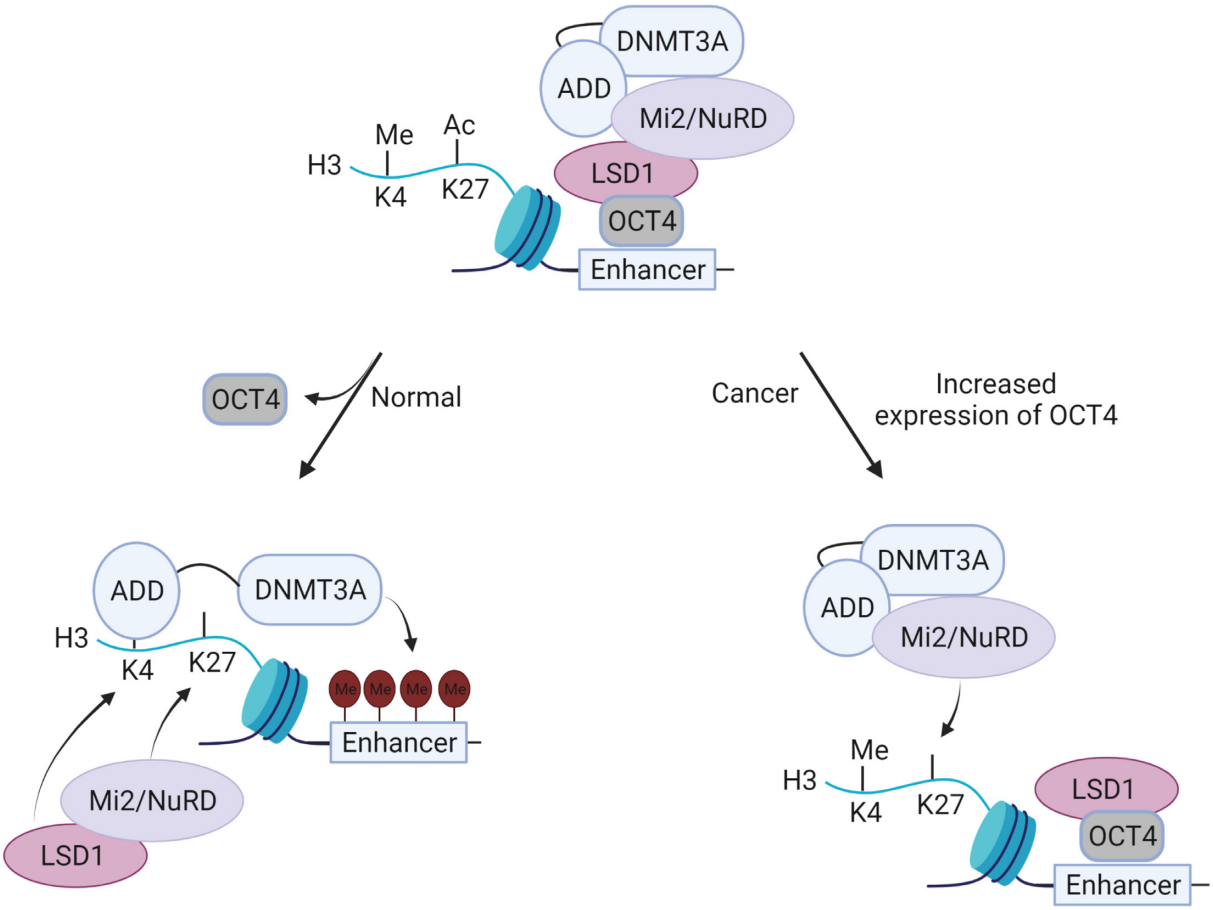


Figure 4. Illustration of cross-talk between DNMT3A and the LSD1-Mi2/NuRD complex in normal versus cancer cells. The ADD domain allosterically inhibits the methyltransferase activity of DNMT3A via direct interaction with the catalytic domain. Inactive DNMT3A interacts with the LSD1/MI2/NuRD complex and is recruited to active enhancers via interactions with transcription factors such as OCT4. Notably, the demethylase activity of LSD1 is inhibited by OCT4, and its loss thereof activates LSD1 to demethylate H3K4. This releases the ADD domain to interact with unmodified H3K4, which relieves the activity of DNMT3A to methylate DNA. The deacetylation of H3K27 by the MI2/NuRD complex in concert with DNA methylation creates a repressive environment that hinders gene expression. However, in cancer cells with elevated OCT4 expression, OCT4 remains bound to enhancer regions and inhibits the activity of LSD1, thus, retaining H3K4 methylation. The methylated H3K4 maintains DNMT3A in an autoinhibited state, preventing its participation in mediating a repressive chromatin environment, leading to spurious expression of genes such as PpGs in cancer.

tion by LSD1–Mi2/NuRD complex facilitates the interaction of DNMT3A-ADD domain with histone H3 leading to subsequent activation of DNA methyltransferase activity at PpG enhancers (254) (Figure 4). These observations are crucial given that about a third of all cancers abnormally express PpGs. A disruption in the enhancer repression mechanism could potentially allow spurious expression of PpGs in cancer. In line with this hypothesis, a subsequent study showed incomplete PpG repression in differentiating embryonal carcinoma cells, owing to a failure in LSD1 and DNMT3A activity at their respective enhancers. Interestingly, it was shown that in differentiating ECC, high OCT4 expression leads to its continued interaction with LSD1 and that OCT4 inhibits LSD1 activity. This inhibits DNA methylation through the retention of H3K4me at the PpG enhancers in ECCs (255).

Besides the canonical histone tail modifications that interact with DNMTs, the association of DNMT1 with ubiquitinated H3 activates its enzymatic activity (256,257). Interaction of DNMTs with modified histone tails has been implicated in cancer. Particularly, DNMTs are enriched at high CpG density class genes in embryonic carcinoma cells (ECC). Notably, this high enrichment of DNMTs correlates with the increased enrichment of H3K27me3, H2AK119ub and H3K36me3 histone modifications in EC cells (258).

CONCLUSIONS/OUTLOOK

Here we review the enzymatic and biological properties of DNMTs, and the effects of their activity in normal and cancer cells. We discuss the known effects of DNMT3A mutations in Acute Myeloid Leukemia in light of the structural and enzymatic properties of DNMT3A and regulation of DNMT function by transcriptional, post-transcriptional mechanisms, and protein–protein interactions.

Our understanding of the regulatory processes that control the activity of DNMTs is far from complete. This is partly because the effect of DNA methylation on gene expression is ‘context’ dependent, and ‘context’ could be defined by DNA sequence, position relative to genes, and chromatin environment. For example, whereas DNA

methylation at gene promoters coincides with gene repression, highly transcribed genes have high DNA methylation in their gene bodies. As a result, loss of DNMT regulation has varying effects in cancer, resulting in both hyper- and hypomethylation of the genome.

DNMTs sustain one of the most well-studied epigenetic regulatory mechanisms, putting them at the forefront of research related to TSG silencing, oncogene expression, and cancer cell proliferation. Expression changes in DNMTs, particularly overexpression of DNMT3B, are reported in many cancers. DNMT expression can be upregulated by diverse factors, including Sp1 and Sp3 zinc finger proteins, Wilms tumor 1, Homeobox B3 and various human viruses (259–261). Both losses of transcriptional repression control and gain of spurious induction have been reported to explain high DNMT expression in cancers. Nucleoside analogs, azacytidine, and decitabine, which target DNMTs are used to treat myeloid malignancies. However, the effectiveness of these drugs is limiting due to their low bioavailability, relative toxicity, and non-specific effects at high doses. (reviewed in (262)). A new class of non-nucleoside drugs targeting DNMTs in cancers is currently being developed to potentially mitigate these challenges (263,264).

Targeting of DNA methylation to regulatory elements is orchestrated by a complex interplay of DNA and chromatin associating factors that associate with DNMTs. A list of factors directly or indirectly interacting with DNMTs is compiled in Table 1. For example, a cross-talk between LSD1/Mi2NuRD complex with DNMT3A at the PpG enhancers ensures deposition of DNA methylation at these sites when the genes are turned off. Disruption of this mechanism leads to incomplete gene repression in embryonal carcinoma cells (255). Similarly, repression of NY-ESO1 gene in glioma and mesothelioma cells occurs through sequential recruitment of three chromatin-modifying complexes: (i) HDAC1/mSIN3a/NCOR complex deacetylates the promoter, (ii) DNMT3B/HDAC1/EGR1 complex establishes site-specific DNA methylation and histone deacetylation, and (iii) DNMT1/PCNA/UHRF1/G9a complex maintains DNA methylation and initiates heterochromatinization by introducing H3K9me2 repressive mark (265). A similar strategy at repetitive elements establishes and maintains heterochromatin, preventing chromosomal aberrations and ensuring centromere maintenance. The process involves cross-talk between DNMT3A/3B with HP1 and H3K9me3 (266). The importance of the discovery of cross-talk mechanisms is emphasized by the success of combination therapies targeting DNMTs and other chromatin-modifying enzymes, such as HDACs in various cancers. (41,267).

Although DNA methylation is pervasive across the mammalian genome, in cancer cells, an aberrant increase in DNA methylation occurs at defined sites, suggesting mistargeting rather than increased enzymatic activity. Therefore, in the simplest model, inhibitors that disrupt the interactions mistargeting DNMTs in cancer cells can potentially prevent aberrant targeting and hypermethylation. For more complex mechanisms, the cascades of regulatory reactions that affect DNMT activity can be modeled to design inhibitors that disrupt DNMT activity during cancer development. A thorough analysis of similarities and differences in the allosteric regulation and structure-function relationship of DNMTs will enable the development of inhibitors that selectively target these enzymes. Furthermore, owing to the tissue-specific expression of DNMTs, their pathways may also vary in different cancers. Understanding the tissue-specific mechanism of DNMTs could potentially lead to an effective cancer-specific therapeutic strategy.

In conclusion, an in-depth understanding of DNMTs, their enzymatic control, interaction partners, chromatin localization, and interaction with various signaling pathways is needed for the discovery of new cancer drug targets and to deepen our understanding of mechanisms that define cell identity.

FUNDING

National Institutes of Health (NIH) [R01GM118654-01].
Conflict of interest statement. None declared.

REFERNENCES

1. Zemach,A., McDaniel,I.E., Silva,P. and Zilberman,D. (2010) Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science*, **328**, 916–919.
2. Edwards,J.R., Yarychkivska,O., Boulard,M. and Bestor,T.H. (2017) DNA methylation and DNA methyltransferases. *Epigenetics Chromatin*, **10**, 23.
3. Greenberg,M.V.C. and Bourc'his,D. (2019) The diverse roles of DNA methylation in mammalian development and disease. *Nat. Rev. Mol. Cell Biol.*, **20**, 590–607.
4. Li,E. and Zhang,Y. (2014) DNA methylation in mammals. *Cold Spring Harb. Perspect. Biol.*, **6**, a019133.
5. Baylin,S.B. and Jones,P.A. (2016) Epigenetic determinants of cancer. *Cold Spring Harb. Perspect. Biol.*, **8**, a019505.
6. Jones,P.A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.*, **13**, 484–492.
7. Weber,M., Davies,J.J., Wittig,D., Oakeley,E.J., Haase,M., Lam,W.L. and Schübeler,D. (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat. Genet.*, **37**, 853–862.
8. Lister,R., Pelizzola,M., Dowen,R.H., Hawkins,R.D., Hon,G., Tonti-Filippini,J., Nery,J.R., Lee,L., Ye,Z., Ngo,Q.M. *et al.* (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, **462**, 315–322.
9. Zhu,J., He,F., Hu,S. and Yu,J. (2008) On the nature of human housekeeping genes. *Trends Genet.*, **24**, 481–484.
10. Wang,Y. and Leung,F.C.C. (2004) An evaluation of new criteria for CpG islands in the human genome as gene markers. *Bioinformatics*, **20**, 1170–1177.
11. Weber,M., Hellmann,I., Stadler,M.B., Ramos,L., Pääbo,S., Rebhan,M. and Schübeler,D. (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.*, **39**, 457–466.
12. Stadler,M.B., Murr,R., Burger,L., Ivanek,R., Lienert,F., Scholer,A., van Nimwegen,E., Wirbelauer,C., Oakeley,E.J., Gaidatzis,D. *et al.* (2011) DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature*, **480**, 490–495.
13. Saha,D., Norvil,A.B., Lanman,N.A. and Gowher,H. (2020) Simplified MethylRAD sequencing to detect changes in DNA methylation at enhancer elements in differentiating embryonic stem cells. *Epigenomes*, **4**, 24.
14. Luo,C., Keown,C.L., Kurihara,L., Zhou,J., He,Y., Li,J., Castanon,R., Lucero,J., Nery,J.R., Sandoval,J.P. *et al.* (2017) Single-cell methylomes identify neuronal subtypes and regulatory elements in mammalian cortex. *Science*, **357**, 600–604.
15. Ziller,M.J., Gu,H., Muller,F., Donaghey,J., Tsai,L.T., Kohlbacher,O., De Jager,P.L., Rosen,E.D., Bennett,D.A., Bernstein,B.E. *et al.* (2013) Charting a dynamic DNA methylation landscape of the human genome. *Nature*, **500**, 477–481.

16. Aran,D., Sabato,S. and Hellman,A. (2013) DNA methylation of distal regulatory sites characterizes dysregulation of cancer genes. *Genome Biol.*, **14**, R21.
17. Aran,D. and Hellman,A. (2014) Unmasking risk loci: DNA methylation illuminates the biology of cancer predisposition: analyzing DNA methylation of transcriptional enhancers reveals missed regulatory links between cancer risk loci and genes. *Bioessays*, **36**, 184–190.
18. Aran,D. and Hellman,A. (2013) DNA methylation of transcriptional enhancers and cancer predisposition. *Cell*, **154**, 11–13.
19. Jones,P.A., Issa,J.P. and Baylin,S. (2016) Targeting the cancer epigenome for therapy. *Nat. Rev. Genet.*, **17**, 630–641.
20. Guo,X., Wang,L., Li,J., Ding,Z., Xiao,J., Yin,X., He,S., Shi,P., Dong,L., Li,G. *et al.* (2015) Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature*, **517**, 640–644.
21. Petell,C.J., Alabdi,L., He,M., San Miguel,P., Rose,R. and Gowher,H. (2016) An epigenetic switch regulates de novo DNA methylation at a subset of pluripotency gene enhancers during embryonic stem cell differentiation. *Nucleic Acids Res.*, **44**, 7605–7617.
22. Weinberg,D.N., Papillon-Cavanagh,S., Chen,H., Yue,Y., Chen,X., Rajagopalan,K.N., Horth,C., McGuire,J.T., Xu,X., Nikbakht,H. *et al.* (2019) The histone mark H3K36me2 recruits DNMT3A and shapes the intergenic DNA methylation landscape. *Nature*, **573**, 281–286.
23. Ren,W., Fan,H., Grimm,S.A., Kim,J.J., Li,L., Guo,Y., Petell,C.J., Tan,X.-F., Zhang,Z.-M., Coan,J.P. *et al.* (2021) DNMT1 reads heterochromatic H4K20me3 to reinforce LINE-1 DNA methylation. *Nat. Commun.*, **12**, 2490.
24. Smith,Z.D. and Meissner,A. (2013) DNA methylation: roles in mammalian development. *Nat. Rev. Genet.*, **14**, 204–220.
25. Keshet,I., Schlesinger,Y., Farkash,S., Rand,E., Hecht,M., Segal,E., Pikarski,E., Young,R.A., Niveleau,A., Cedar,H. *et al.* (2006) Evidence for an instructive mechanism of de novo methylation in cancer cells. *Nat. Genet.*, **38**, 149–153.
26. Héberlé,É. and Bardet,A.F. (2019) Sensitivity of transcription factors to DNA methylation. *Essays Biochem.*, **63**, 727–741.
27. Hu,S., Wan,J., Su,Y., Song,Q., Zeng,Y., Nguyen,H.N., Shin,J., Cox,E., Rho,H.S., Woodard,C. *et al.* (2013) DNA methylation presents distinct binding sites for human transcription factors. *eLife*, **2**, e00726.
28. Jin,J., Lian,T., Gu,C., Yu,K., Gao,Y.Q. and Su,X.D. (2016) The effects of cytosine methylation on general transcription factors. *Sci. Rep.*, **6**, 29119.
29. Baubec,T., Colombo,D.F., Wirbelauer,C., Schmidt,J., Burger,L., Krebs,A.R., Akalin,A. and Schubeler,D. (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature*, **520**, 243–247.
30. Morselli,M., Pastor,W.A., Montanini,B., Nee,K., Ferrari,R., Fu,K., Bonora,G., Rubbi,L., Clark,A.T., Ottonello,S. *et al.* (2015) In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. *Elife*, **4**, e06205.
31. Wu,H., Coskun,V., Tao,J., Xie,W., Ge,W., Yoshikawa,K., Li,E., Zhang,Y. and Sun,Y.E. (2010) Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science*, **329**, 444–448.
32. Neri,F., Rapelli,S., Krepelova,A., Incarnato,D., Parlato,C., Basile,G., Maldotti,M., Anselmi,F. and Oliviero,S. (2017) Intragenic DNA methylation prevents spurious transcription initiation. *Nature*, **543**, 72–77.
33. Teissandier,A. and Bourc'his,D. (2017) Gene body DNA methylation conspires with H3K36me3 to preclude aberrant transcription. *EMBO J.*, **36**, 1471–1473.
34. Gelfman,S., Cohen,N., Yearim,A. and Ast,G. (2013) DNA-methylation effect on cotranscriptional splicing is dependent on GC architecture of the exon-intron structure. *Genome Res.*, **23**, 789–799.
35. Shayevitch,R., Askayo,D., Keydar,I. and Ast,G. (2018) The importance of DNA methylation of exons on alternative splicing. *RNA*, **24**, 1351–1362.
36. Yang,X.J., Han,H., De Carvalho,D.D., Lay,F.D., Jones,P.A. and Liang,G.N. (2014) Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell*, **26**, 577–590.
37. Meehan,R.R., Lewis,J.D., McKay,S., Kleiner,E.L. and Bird,A.P. (1989) Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. *Cell*, **58**, 499–507.
38. Baubec,T., Ivanek,R., Lienert,F. and Schubeler,D. (2013) Methylation-dependent and -independent genomic targeting principles of the MBD protein family. *Cell*, **153**, 480–492.
39. Myant,K., Termanis,A., Sundaram,A.Y., Boe,T., Li,C., Merusi,C., Burrage,J., de Las Heras,J.I. and Stancheva,I. (2011) LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. *Genome Res.*, **21**, 83–94.
40. Du,Q., Luu,P.L., Stirzaker,C. and Clark,S.J. (2015) Methyl-CpG-binding domain proteins: readers of the epigenome. *Epigenomics*, **7**, 1051–1073.
41. Cheishvili,D., Boureau,L. and Szyf,M. (2015) DNA demethylation and invasive cancer: implications for therapeutics. *Br. J. Pharmacol.*, **172**, 2705–2715.
42. Ateeq,B., Unterberger,A., Szyf,M. and Rabbani,S.A. (2008) Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes in vitro and in vivo. *Neoplasia*, **10**, 266–278.
43. Yu,Y., Zeng,P., Xiong,J., Liu,Z., Berger,S.L. and Merlino,G. (2010) Epigenetic drugs can stimulate metastasis through enhanced expression of the pro-metastatic Ezrin gene. *PLoS One*, **5**, e12710.
44. Chik,F. and Szyf,M. (2011) Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. *Carcinogenesis*, **32**, 224–232.
45. Goll,M.G. and Bestor,T.H. (2005) Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.*, **74**, 481–514.
46. Hermann,A., Gowher,H. and Jeltsch,A. (2004) Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci.*, **61**, 2571–2587.
47. Okano,M., Bell,D.W., Haber,D.A. and Li,E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, **99**, 247–257.
48. Chédin,F. (2011) In: Cheng,X. and Blumenthal,R.M. (eds). *Progress in Molecular Biology and Translational Science*. Academic Press, Vol. **101**, pp. 255–285.
49. Okano,M., Takebayashi,S., Okumura,K. and Li,E. (1999) Assignment of cytosine-5 DNA methyltransferases Dnmt3a and Dnmt3b to mouse chromosome bands 12A2-A3 and 2H1 by in situ hybridization. *Cytogenet. Cell Genet.*, **86**, 333–334.
50. Jones,P.A. and Liang,G. (2009) Rethinking how DNA methylation patterns are maintained. *Nat. Rev. Genet.*, **10**, 805–811.
51. Li,E., Bestor,T.H. and Jaenisch,R. (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, **69**, 915–926.
52. Liao,J., Karnik,R., Gu,H.C., Ziller,M.J., Clement,K., Tsankov,A.M., Akopian,V., Gifford,C.A., Donaghey,J., Galonska,C. *et al.* (2015) Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. *Nat. Genet.*, **47**, 469–478.
53. Chen,T.P., Hevi,S., Gay,F., Tsujimoto,N., He,T., Zhang,B.L., Ueda,Y. and Li,E. (2007) Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. *Nat. Genet.*, **39**, 391–396.
54. Svedružić,Ž.M. (2011) In: Cheng,X. and Blumenthal,R.M. (eds). *Progress in Molecular Biology and Translational Science*. Academic Press, Vol. **101**, pp. 221–254.
55. Jurkowska,R.Z., Jurkowski,T.P. and Jeltsch,A. (2011) Structure and function of mammalian DNA methyltransferases. *Chembiochem*, **12**, 206–222.
56. Tajima,S., Suetake,I., Takeshita,K., Nakagawa,A. and Kimura,H. (2016) Domain Structure of the Dnmt1, Dnmt3a, and Dnmt3b DNA Methyltransferases. *DNA Methyltransferases - Role Funct.*, **945**, 63–86.
57. Chuang,L.S.H., Ian,H.I., Koh,T.W., Ng,H.H., Xu,G.L. and Li,B.F.L. (1997) Human DNA (cytosine-5) methyltransferase PCNA complex as a target for p21(WAF1). *Science*, **277**, 1996–2000.
58. Esteve,P.O., Chin,H.G., Smallwood,A., Feehery,G.R., Gangisetty,O., Karpf,A.R., Carey,M.F. and Pradhan,S. (2006) Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Gene Dev*, **20**, 3089–3103.

59. Fuks,F., Hurd,P.J., Deplus,R. and Kouzarides,T. (2003) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res.*, **31**, 2305–2312.
60. Fellinger,K., Rothbauer,U., Felle,M., Langst,G. and Leonhardt,H. (2009) Dimerization of DNA methyltransferase 1 is mediated by its regulatory domain. *J. Cell. Biochem.*, **106**, 521–528.
61. Syeda,F., Fagan,R.L., Wean,M., Avvakumov,G.V., Walker,J.R., Xue,S., Dhe-Paganon,S. and Brenner,C. (2011) The replication focus targeting sequence (RFTS) domain is a DNA-competitive inhibitor of Dnmt1. *J. Biol. Chem.*, **286**, 15344–15351.
62. Takeshita,K., Suetake,I., Yamashita,E., Suga,M., Narita,H., Nakagawa,A. and Tajima,S. (2011) Structural insight into maintenance methylation by mouse DNA methyltransferase 1 (Dnmt1). *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 9055–9059.
63. Bostick,M., Kim,J.K., Esteve,P.O., Clark,A., Pradhan,S. and Jacobsen,S.E. (2007) UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science*, **317**, 1760–1764.
64. Ren,W., Fan,H., Grimm,S.A., Guo,Y., Kim,J.J., Yin,J., Li,L., Petell,C.J., Tan,X.F., Zhang,Z.M. *et al.* (2020) Direct readout of heterochromatic H3K9me3 regulates DNMT1-mediated maintenance DNA methylation. *Proc. Natl. Acad. Sci. U.S.A.*, **117**, 18439–18447.
65. Song,J., Rechkoblit,O., Bestor,T.H. and Patel,D.J. (2011) Structure of DNMT1-DNA complex reveals a role for autoinhibition in maintenance DNA methylation. *Science*, **331**, 1036–1040.
66. Yarychkivska,O., Shahabuddin,Z., Comfort,N., Boulard,M. and Bestor,T.H. (2018) BAH domains and a histone-like motif in DNA methyltransferase 1 (DNMT1) regulate de novo and maintenance methylation in vivo. *J. Biol. Chem.*, **293**, 19466–19475.
67. Hermann,A., Goyal,R. and Jeltsch,A. (2004) The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J. Biol. Chem.*, **279**, 48350–48359.
68. Vilkaitis,G., Suetake,I., Klimasauskas,S. and Tajima,S. (2005) Processive methylation of hemimethylated CpG sites by mouse Dnmt1 DNA methyltransferase. *J. Biol. Chem.*, **280**, 64–72.
69. Feltus,F.A., Lee,E.K., Costello,J.F., Plass,C. and Vertino,P.M. (2003) Predicting aberrant CpG island methylation. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 12253–12258.
70. Jair,K.W., Bachman,K.E., Suzuki,H., Ting,A.H., Rhee,I., Yen,R.W., Baylin,S.B. and Schuebel,K.E. (2006) De novo CpG island methylation in human cancer cells. *Cancer Res.*, **66**, 682–692.
71. Haggerty,C., Kretzmer,H., Riemenschneider,C., Kumar,A.S., Mattei,A.L., Bailly,N., Gottfreund,J., Giesselmann,P., Weigert,R., Brandl,B. *et al.* (2021) Dnmt1 has de novo activity targeted to transposable elements. *Nat. Struct. Mol. Biol.*, **28**, 594–603 .
72. Pradhan,S., Bacolla,A., Wells,R.D. and Roberts,R.J. (1999) Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. *J. Biol. Chem.*, **274**, 33002–33010.
73. Fatemi,M., Hermann,A., Gowher,H. and Jeltsch,A. (2002) Dnmt3a and Dnmt1 functionally cooperate during de novo methylation of DNA. *Eur. J. Biochem.*, **269**, 4981–4984.
74. Deplus,R., Brenner,C., Burgers,W.A., Putmans,P., Kouzarides,T., de Launoit,Y. and Fuks,F. (2002) Dnmt3L is a transcriptional repressor that recruits histone deacetylase. *Nucleic Acids Res.*, **30**, 3831–3838.
75. Watanabe,D., Suetake,I., Tada,T. and Tajima,S. (2002) Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis. *Mech. Dev.*, **118**, 187–190.
76. Okano,M., Bell,D.W., Haber,D.A. and Li,E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, **99**, 247–257.
77. Schubeler,D. (2015) Function and information content of DNA methylation. *Nature*, **517**, 321–326.
78. Aapola,U., Kawasaki,K., Scott,H.S., Ollila,J., Vihinen,M., Heino,M., Shintani,A., Kawasaki,K., Minoshima,S., Krohn,K. *et al.* (2000) Isolation and initial characterization of a novel zinc finger gene, DNMT3L, on 21q22.3, related to the cytosine-5-methyltransferase 3 gene family. *Genomics*, **65**, 293–298.
79. Aapola,U., Lyle,R., Krohn,K., Antonarakis,S.E. and Peterson,P. (2001) Isolation and initial characterization of the mouse Dnmt3l gene. *Cytogenet. Cell Genet.*, **92**, 122–126.
80. Chedin,F., Lieber,M.R. and Hsieh,C.L. (2002) The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 16916–16921.
81. Lees-Murdock,D.J., Shovlin,T.C., Gardiner,T., De Felici,M. and Walsh,C.P. (2005) DNA methyltransferase expression in the mouse germ line during periods of de novo methylation. *Dev. Dyn.*, **232**, 992–1002.
82. La Salle,S., Mertineit,C., Taketo,T., Moens,P.B., Bestor,T.H. and Trasler,J.M. (2004) Windows for sex-specific methylation marked by DNA methyltransferase expression profiles in mouse germ cells. *Dev. Biol.*, **268**, 403–415.
83. Bourc'his,D., Xu,G.L., Lin,C.S., Bollman,B. and Bestor,T.H. (2001) Dnmt3L and the establishment of maternal genomic imprints. *Science*, **294**, 2536–2539.
84. Hata,K., Okano,M., Lei,H. and Li,E. (2002) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development*, **129**, 1983–1993.
85. Kaneda,M., Okano,M., Hata,K., Sado,T., Tsujimoto,N., Li,E. and Sasaki,H. (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature*, **429**, 900–903.
86. Webster,K.E., O'Bryan,M.K., Fletcher,S., Crewther,P.E., Aapola,U., Craig,J., Harrison,D.K., Aung,H., Phutikanit,N., Lyle,R. *et al.* (2005) Meiotic and epigenetic defects in Dnmt3L-knockout mouse spermatogenesis. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 4068–4073.
87. Cheng,X. and Blumenthal,R.M. (2008) Mammalian DNA methyltransferases: a structural perspective. *Structure*, **16**, 341–350.
88. Chen,T., Tsujimoto,N. and Li,E. (2004) The PWWP domain of Dnmt3a and Dnmt3b is required for directing DNA methylation to the major satellite repeats at pericentric heterochromatin. *Mol. Cell. Biol.*, **24**, 9048–9058.
89. Dhayalan,A., Rajavelu,A., Rathert,P., Tamas,R., Jurkowska,R.Z., Ragozin,S. and Jeltsch,A. (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J. Biol. Chem.*, **285**, 26114–26120.
90. Wu,H., Zeng,H., Lam,R., Tempel,W., Amaya,M.F., Xu,C., Dombrovski,L., Qiu,W., Wang,Y. and Min,J. (2011) Structural and histone binding ability characterizations of human PWWP domains. *PLoS One*, **6**, e18919.
91. Weinberg,D.N., Rosenbaum,P., Chen,X., Barrows,D., Horth,C., Marunde,M.R., Popova,I.K., Gillespie,Z.B., Keogh,M.C., Lu,C. *et al.* (2021) Two competing mechanisms of DNMT3A recruitment regulate the dynamics of de novo DNA methylation at PRC1-targeted CpG islands. *Nat. Genet.*, **53**, 794–800.
92. Ooi,S.K., Qiu,C., Bernstein,E., Li,K., Jia,D., Yang,Z., Erdjument-Bromage,H., Tempst,P., Lin,S.P., Allis,C.D. *et al.* (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*, **448**, 714–717.
93. Otani,J., Nankumo,T., Arita,K., Inamoto,S., Ariyoshi,M. and Shirakawa,M. (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep.*, **10**, 1235–1241.
94. Kumar,S., Cheng,X., Klimasauskas,S., Mi,S., Posfai,J., Roberts,R.J. and Wilson,G.G. (1994) The DNA (cytosine-5) methyltransferases. *Nucleic Acids Res.*, **22**, 1–10.
95. Jeltsch,A. (2002) Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases. *Chembiochem*, **3**, 274–293.
96. Gowher,H. and Jeltsch,A. (2002) Molecular enzymology of the catalytic domains of the Dnmt3a and Dnmt3b DNA methyltransferases. *J. Biol. Chem.*, **277**, 20409–20414.
97. Gowher,H., Liebert,K., Hermann,A., Xu,G. and Jeltsch,A. (2005) Mechanism of stimulation of catalytic activity of Dnmt3A and Dnmt3B DNA-(cytosine-C5)-methyltransferases by Dnmt3L. *J. Biol. Chem.*, **280**, 13341–13348.
98. Jia,D., Jurkowska,R.Z., Zhang,X., Jeltsch,A. and Cheng,X. (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature*, **449**, 248–251.
99. Holz-Schietinger,C., Matje,D.M., Harrison,M.F. and Reich,N.O. (2011) Oligomerization of DNMT3A controls the mechanism of de novo DNA methylation. *J. Biol. Chem.*, **286**, 41479–41488.
100. Jurkowska,R.Z., Rajavelu,A., Anspach,N., Urbanke,C., Jankevicius,G., Ragozin,S., Nellen,W. and Jeltsch,A. (2011) Oligomerization and binding of the Dnmt3a DNA

methyltransferase to parallel DNA molecules: heterochromatic localization and role of Dnmt3L. *J. Biol. Chem.*, **286**, 24200–24207.
101. Holz-Schietinger,C., Matje,D.M. and Reich,N.O. (2012) Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. *J. Biol. Chem.*, **287**, 30941–30951.
102. Gowher,H., Loutchanwoot,P., Vorobjeva,O., Handa,V., Jurkowska,R.Z., Jurkowski,T.P. and Jeltsch,A. (2006) Mutational analysis of the catalytic domain of the murine Dnmt3a DNA-(cytosine C5)-methyltransferase. *J. Mol. Biol.*, **357**, 928–941.
103. Russler-Germain,D.A., Spencer,D.H., Young,M.A., Lamprecht,T.L., Miller,C.A., Fulton,R., Meyer,M.R., Erdmann-Gilmore,P., Townsend,R.R., Wilson,R.K. *et al.* (2014) The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell*, **25**, 442–454.
104. Norvil,A.B., Petell,C.J., Alabdi,L., Wu,L., Rossie,S. and Gowher,H. (2018) Dnmt3b methylates DNA by a noncooperative mechanism, and its activity is unaffected by manipulations at the predicted dimer interface. *Biochemistry*, **57**, 4312–4324.
105. Lin,C.C., Chen,Y.P., Yang,W.Z., Shen,J.C.K. and Yuan,H.S. (2020) Structural insights into CpG-specific DNA methylation by human DNA methyltransferase 3B. *Nucleic Acids Res.*, **48**, 3949–3961.
106. Xu,T.-H., Liu,M., Zhou,X.E., Liang,G., Zhao,G., Xu,H.E., Melcher,K. and Jones,P.A. (2020) Structure of nucleosome-bound DNA methyltransferases DNMT3A and DNMT3B. *Nature*, **586**, 151–155.
107. Jones,P.A. and Liang,G. (2009) Rethinking how DNAmethylation patterns are maintained. *Nat. Rev. Genet.*, **10**, 805–811.
108. Han,M., Jia,L.N., Lv,W.C., Wang,L.H. and Cui,W. (2019) Epigenetic enzyme mutations: role in tumorigenesis and molecular inhibitors. *Front. Oncol.*, **9**, 194.
109. Kanai,Y., Ushijima,S., Nakanishi,Y., Sakamoto,M. and Hirohashi,S. (2003) Mutation of the DNA methyltransferase (DNMT) 1 gene in human colorectal cancers. *Cancer Lett.*, **192**, 75–82.
110. Hansen,R.S., Wijmenga,C., Luo,P., Stanek,A.M., Canfield,T.K., Weemaes,C.M. and Gartler,S.M. (1999) The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 14412–14417.
111. Ley,T.J., Ding,L., Walter,M.J., McLellan,M.D., Lamprecht,T., Larson,D.E., Kandoth,C., Payton,J.E., Baty,J., Welch,J. *et al.* (2010) DNMT3A mutations in acute myeloid leukemia. *N. Engl. J. Med.*, **363**, 2424–2433.
112. Challen,G.A., Sun,D.Q., Jeong,M., Luo,M., Jelinek,J., Berg,J.S., Bock,C., Vasanthakumar,A., Gu,H.C., Xi,Y.X. *et al.* (2012) Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.*, **44**, 23–U43.
113. Jeong,M., Park,H.J., Celik,H., Ostrander,E.L., Reyes,J.M., Guzman,A., Rodriguez,B., Lei,Y., Lee,Y., Ding,L. *et al.* (2018) Loss of Dnmt3a immortalizes hematopoietic stem cells in vivo. *Cell Rep.*, **23**, 1–10.
114. Challen,G.A., Sun,D., Mayle,A., Jeong,M., Luo,M., Rodriguez,B., Mallaney,C., Celik,H., Yang,L., Xia,Z. *et al.* (2014) Dnmt3a and Dnmt3b have overlapping and distinct functions in hematopoietic stem cells. *Cell Stem Cell*, **15**, 350–364.
115. Brunetti,L., Gundry,M.C. and Goodell,M.A. (2017) DNMT3A in leukemia. *Cold Spring Harb. Perspect. Med.*, **7**, a030320.
116. Yang,L., Rau,R. and Goodell,M.A. (2015) DNMT3A in haematological malignancies. *Nat. Rev. Cancer*, **15**, 152–165.
117. Yan,X.J., Xu,J., Gu,Z.H., Pan,C.M., Lu,G., Shen,Y., Shi,J.Y., Zhu,Y.M., Tang,L., Zhang,X.W. *et al.* (2011) Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nat. Genet.*, **43**, 309–315.
118. Jankowska,A.M., Makishima,H., Tiu,R.V., Szpurka,H., Huang,Y., Traina,F., Visconte,V., Sugimoto,Y., Prince,C., O'Keefe,C. *et al.* (2011) Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. *Blood*, **118**, 3932–3941.
119. Park,D.J., Kwon,A., Cho,B.S., Kim,H.J., Hwang,K.A., Kim,M. and Kim,Y. (2020) Characteristics of DNMT3A mutations in acute myeloid leukemia. *Blood Res*, **55**, 17–26.
120. Gale,R.E., Lamb,K., Allen,C., El-Sharkawi,D., Stowe,C., Jenkinson,S., Tinsley,S., Dickson,G., Burnett,A.K., Hills,R.K. *et al.* (2015) Simpson's paradox and the impact of different DNMT3A mutations on outcome in younger adults with acute myeloid leukemia. *J. Clin. Oncol.*, **33**, 2072–2083.
121. Yuan,X.Q., Chen,P., Du,Y.X., Zhu,K.W., Zhang,D.Y., Yan,H., Liu,H., Liu,Y.L., Cao,S., Zhou,G. *et al.* (2019) Influence of DNMT3A R882 mutations on AML prognosis determined by the allele ratio in Chinese patients. *J. Transl. Med.*, **17**, 220.
122. Kim,S.J., Zhao,H., Hardikar,S., Singh,A.K., Goodell,M.A. and Chen,T. (2013) A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells. *Blood*, **122**, 4086–4089.
123. Emperle,M., Rajavelu,A., Kunert,S., Arimondo,P.B., Reinhardt,R., Jurkowska,R.Z. and Jeltsch,A. (2018) The DNMT3A R882H mutant displays altered flanking sequence preferences. *Nucleic Acids Res.*, **46**, 3130–3139.
124. Norvil,A.B., AlAbdi,L., Liu,B.G., Tu,Y.H., Forstoffer,N.E., Michie,A.R., Chen,T.P. and Gowher,H. (2020) The acute myeloid leukemia variant DNMT3A Arg882His is a DNMT3B-like enzyme. *Nucleic Acids Res.*, **48**, 3761–3775.
125. Emperle,M., Dukatz,M., Kunert,S., Holzer,K., Rajavelu,A., Jurkowska,R.Z. and Jeltsch,A. (2018) The DNMT3A R882H mutation does not cause dominant negative effects in purified mixed DNMT3A/R882H complexes. *Sci. Rep.*, **8**, 13242.
126. Emperle,M., Adam,S., Kunert,S., Dukatz,M., Baude,A., Plass,C., Rathert,P., Bashtrykov,P. and Jeltsch,A. (2019) Mutations of R882 change flanking sequence preferences of the DNA methyltransferase DNMT3A and cellular methylation patterns. *Nucleic Acids Res.*, **47**, 11355–11367.
127. Kohlmann,A., Schoch,C., Dugas,M., Schnittger,S., Hiddemann,W., Kern,W. and Haferlach,T. (2005) New insights into MLL gene rearranged acute leukemias using gene expression profiling: shared pathways, lineage commitment, and partner genes. *Leukemia*, **19**, 953–964.
128. Ferreira,H.J., Heyn,H., Vizoso,M., Moutinho,C., Vidal,E., Gomez,A., Martinez-Cardus,A., Simo-Riudalbas,L., Moran,S., Jost,E. *et al.* (2016) DNMT3A mutations mediate the epigenetic reactivation of the leukemogenic factor MEIS1 in acute myeloid leukemia. *Oncogene*, **35**, 3079–3082.
129. Xu,J., Wang,Y.Y., Dai,Y.J., Zhang,W., Zhang,W.N., Xiong,S.M., Gu,Z.H., Wang,K.K., Zeng,R., Chen,Z. *et al.* (2014) DNMT3A Arg882 mutation drives chronic myelomonocytic leukemia through disturbing gene expression/DNA methylation in hematopoietic cells. *Proc. Natl. Acad. Sci. U.S.A.*, **111**, 2620–2625.
130. Dai,Y.J., Wang,Y.Y., Huang,J.Y., Xia,L., Shi,X.D., Xu,J., Lu,J., Su,X.B., Yang,Y., Zhang,W.N. *et al.* (2017) Conditional knockin of Dnmt3a R878H initiates acute myeloid leukemia with mTOR pathway involvement. *Proc. Natl. Acad. Sci. U.S.A.*, **114**, 5237–5242.
131. Sandoval,J.E., Huang,Y.H., Muise,A., Goodell,M.A. and Reich,N.O. (2019) Mutations in the DNMT3A DNA methyltransferase in acute myeloid leukemia patients cause both loss and gain of function and differential regulation by protein partners. *J. Biol. Chem.*, **294**, 4898–4910.
132. Chen,R.Z., Pettersson,U., Beard,C., Jackson-Grusby,L. and Jaenisch,R. (1998) DNA hypomethylation leads to elevated mutation rates. *Nature*, **395**, 89–93.
133. Oghamian,S., Sodir,N.M., Bashir,M.U., Shen,H., Cullins,A.E., Carroll,C.A., Kundu,P., Shibata,D. and Laird,P.W. (2011) Reduction of pancreatic acinar cell tumor multiplicity in Dnmt1 hypomorphic mice. *Carcinogenesis*, **32**, 829–835.
134. Linhart,H.G., Lin,H., Yamada,Y., Moran,E., Steine,E.J., Gokhale,S., Lo,G., Cantu,E., Ehrich,M., He,T. *et al.* (2007) Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing. *Gene Dev*, **21**, 3110–3122.
135. Deng,T., Kuang,Y., Wang,L., Li,J., Wang,Z.G. and Fei,J. (2009) An essential role for DNA methyltransferase 3a in melanoma tumorigenesis. *Biochem. Biophys. Res. Commun.*, **387**, 611–616.
136. Gao,Q., Steine,E.J., Barrasa,M.I., Hockemeyer,D., Pawlak,M., Fu,D.D., Reddy,S., Bell,G.W. and Jaenisch,R. (2011) Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 18061–18066.
137. Husni,R.E., Shiba-Ishii,A., Iiyama,S., Shiozawa,T., Kim,Y., Nakagawa,T., Sato,T., Kano,J., Minami,Y. and Noguchi,M. (2016) DNMT3a expression pattern and its prognostic value in lung adenocarcinoma. *Lung Cancer*, **97**, 59–65.

138. Haney,S.L., Upchurch,G.M., Opavska,J., Klinkebiel,D., Hlady,R.A., Roy,S., Dutta,S., Datta,K. and Opavsky,R. (2016) Dnmt3a is a haploinsufficient tumor suppressor in CD8+Peripheral T cell lymphoma. *PLoS Genet.*, **12**, e1006334.
139. Zheng,Y., Zhang,H., Wang,Y., Li,X., Lu,P., Dong,F., Pang,Y., Ma,S., Cheng,H., Hao,S. *et al.* (2016) Loss of Dnmt3b accelerates MLL-AF9 leukemia progression. *Leukemia*, **30**, 2373–2384.
140. Jones,P.A. and Gonzalgo,M.L. (1997) Altered DNA methylation and genome instability: A new pathway to cancer? *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 2103–2105.
141. Ehrlich,M. (2002) DNA methylation in cancer: too much, but also too little. *Oncogene*, **21**, 5400–5413.
142. Nishiyama,A. and Nakanishi,M. (2021) Navigating the DNA methylation landscape of cancer. *Trends Genet.*, **37**, 1012–1027.
143. Eads,C.A., Danenberg,K.D., Kawakami,K., Saltz,L.B., Danenberg,P.V. and Laird,P.W. (1999) CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. *Cancer Res.*, **59**, 2302–2306.
144. Subramaniam,D., Thombre,R., Dhar,A. and Anant,S. (2014) DNA methyltransferases: a novel target for prevention and therapy. *Front. Oncol.*, **4**, 80.
145. Zhang,W. and Xu,J. (2017) DNA methyltransferases and their roles in tumorigenesis. *Biomark. Res.*, **5**, 1–1.
146. Nosho,K., Shima,K., Irahara,N., Kure,S., Baba,Y., Kirkner,G.J., Chen,L., Gokhale,S., Hazra,A., Spiegelman,D. *et al.* (2009) DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer. *Clin. Cancer Res.*, **15**, 3663–3671.
147. Kanai,Y., Ushijima,S., Kondo,Y., Nakanishi,Y. and Hirohashi,S. (2001) DNA methyltransferase expression and DNA methylation of CPG islands and peri-centromeric satellite regions in human colorectal and stomach cancers. *Int. J. Cancer*, **91**, 205–212.
148. Ibrahim,A.E.K., Arends,M.J., Silva,A.L., Wyllie,A.H., Greger,L., Ito,Y., Vowler,S.L., Huang,T.H.M., Tavare,S., Murrell,A. *et al.* (2011) Sequential DNA methylation changes are associated with DNMT3B overexpression in colorectal neoplastic progression. *Gut*, **60**, 499–508.
149. Ehrlich,M. (2009) DNA hypomethylation in cancer cells. *Epigenomics-UK*, **1**, 239–259.
150. Wong,N., Lam,W.C., Lai,P.B.S., Pang,E., Lau,W.Y. and Johnson,P.J. (2001) Hypomethylation of chromosome 1 heterochromatin DNA correlates with q-arm copy gain in human hepatocellular carcinoma. *Am. J. Pathol.*, **159**, 465–471.
151. Schulz,W.A., Elo,J.P., Florl,A.R., Pennanen,S., Santourlidis,S., Engers,R., Buchardt,M., Seifert,H.H. and Visakorpi,T. (2002) Genomewide DNA hypomethylation is associated with alterations on chromosome 8 in prostate carcinoma. *Gene Chromosome Canc*, **35**, 58–65.
152. Sheaffer,K.L., Elliott,E.N. and Kaestner,K.H. (2016) DNA hypomethylation contributes to genomic instability and intestinal cancer initiation. *Cancer Prev. Res.*, **9**, 534–546.
153. Cui,H., Zhao,C.C., Gong,P.H., Wang,L., Wu,H.Z., Zhang,K., Zhou,R.P., Wang,L., Zhang,T., Zhong,S. *et al.* (2015) DNA methyltransferase 3A promotes cell proliferation by silencing CDK inhibitor p18(INK4C) in gastric carcinogenesis. *Sci. Rep.*, **5**, 13781.
154. Zhao,Z.J., Wu,Q.X., Cheng,J.A., Qiu,X.M., Zhang,J.Q. and Fan,H. (2010) Depletion of DNMT3A suppressed cell proliferation and restored PTEN in hepatocellular carcinoma cell. *J. Biomed. Biotechnol.* **2010**, 737535.
155. Lin,R.K., Hsu,H.S., Chang,J.W., Chen,C.Y., Chen,J.T. and Wang,Y.C. (2007) Alteration of DNA methyltransferases contributes to 5′CpG methylation and poor prognosis in lung cancer. *Lung Cancer*, **55**, 205–213.
156. Gui,T., Liu,M., Yao,B., Jiang,H.Q., Yang,D.J., Li,Q.X., Zeng,X.W., Wang,Y., Cao,J., Deng,Y.X. *et al.* (2021) TCF3 is epigenetically silenced by EZH2 and DNMT3B and functions as a tumor suppressor in endometrial cancer. *Cell Death Differ*. https://doi.org/10.1038/s41418-021-00824-w.
157. Su,J., Huang,Y.H., Cui,X., Wang,X., Zhang,X., Lei,Y., Xu,J., Lin,X., Chen,K., Lv,J. *et al.* (2018) Homeobox oncogene activation by pan-cancer DNA hypermethylation. *Genome Biol.*, **19**, 108.
158. Arechederra,M., Daian,F., Yim,A., Bazai,S.K., Richelme,S., Dono,R., Saurin,A.J., Habermann,B.H. and Maina,F. (2018) Hypermethylation of gene body CpG islands predicts high dosage of functional oncogenes in liver cancer. *Nat. Commun.*, **9**, 3164.
159. McGuire,M.H., Dasari,S.K., Yao,H., Wen,Y., Mangala,L.S., Bayraktar,E., Ma,W., Ivan,C., Shoshan,E., Wu,S.Y. *et al.* (2021) Gene body methylation of the lymphocyte-specific gene CARD11 results in its overexpression and regulates cancer mTOR signaling. *Mol. Cancer Res.* **19**, 1917–1928.
160. Morita,R., Hirohashi,Y., Suzuki,H., Takahashi,A., Tamura,Y., Kanaseki,T., Asanuma,H., Inoda,S., Kondo,T., Hashino,S. *et al.* (2013) DNA methyltransferase 1 is essential for initiation of the colon cancers. *Exp. Mol. Pathol.*, **94**, 322–329.
161. Broske,A.M., Vockentanz,L., Kharazi,S., Huska,M.R., Mancini,E., Scheller,M., Kuhl,C., Enns,A., Prinz,M., Jaenisch,R. *et al.* (2009) DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat. Genet.*, **41**, 1207–1215.
162. Pathania,R., Ramachandran,S., Elangovan,S., Padia,R., Yang,P.Y., Cinghu,S., Veeranan-Karmegam,R., Arjunan,P., Gnana-Prakasam,J.P., Sadanand,F. *et al.* (2015) DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nat. Commun.*, **6**, 6910.
163. Ahmadnejad,M., Amirizadeh,N., Mehrasa,R., Karkhah,A., Nikougoftar,M. and Oodi,A. (2017) Elevated expression of DNMT1 is associated with increased expansion and proliferation of hematopoietic stem cells co-cultured with human MSCs. *Blood Research*, **52**, 25–30.
164. Robaina,M.C., Mazzoccoli,L., Arruda,V.O., Reis,F.R.D., Apa,A.G., de Rezende,L.M.M. and Klumb,C.E. (2015) Deregulation of DNMT1, DNMT3B and miR-29s in Burkitt lymphoma suggests novel contribution for disease pathogenesis. *Exp. Mol. Pathol.*, **98**, 200–207.
165. Minami,K., Chano,T., Kawakami,T., Ushida,H., Kushima,R., Okabe,H., Okada,Y. and Okamoto,K. (2010) DNMT3L is a novel marker and is essential for the growth of human embryonal carcinoma. *Clin. Cancer Res.*, **16**, 2751–2759.
166. Purkait,S., Sharma,V., Kumar,A., Pathak,P., Mallick,S., Jha,P., Sharma,M.C., Suri,V., Julka,P.K., Suri,A. *et al.* (2016) Expression of DNA methyltransferases 1 and 3B correlates with EZH2 and this 3-marker epigenetic signature predicts outcome in glioblastomas. *Exp. Mol. Pathol.*, **100**, 312–320.
167. Rajendran,G., Shanmuganandam,K., Bendre,A., Mujumdar,D., Goel,A. and Shiras,A. (2011) Epigenetic regulation of DNA methyltransferases: DNMT1 and DNMT3B in gliomas. *J Neuro-Oncol*, **104**, 483–494.
168. Hayette,S., Thomas,X., Jallades,L., Chabane,K., Charlot,C., Tigaud,I., Gazzo,S., Morisset,S., Cornillet-Lefebvre,P., Plesa,A. *et al.* (2012) High DNA methyltransferase DNMT3B levels: a poor prognostic marker in acute myeloid leukemia. *PLoS One*, **7**, e51527.
169. Renneville,A., Boissel,N., Nibourel,O., Berthon,C., Helevaut,N., Gardin,C., Cayuela,J.M., Hayette,S., Reman,O., Contentin,N. *et al.* (2012) Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. *Leukemia*, **26**, 1247–1254.
170. Thol,F., Damm,F., Ludeking,A., Winschel,C., Wagner,K., Morgan,M., Yun,H., Gohring,G., Schlegelberger,B., Hoelzer,D. *et al.* (2011) Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. *J. Clin. Oncol.*, **29**, 2889–2896.
171. Wang,W., Gao,J., Man,X.H., Li,Z.S. and Gong,Y.F. (2009) Significance of DNA methyltransferase-1 and histone deacetylase-1 in pancreatic cancer. *Oncol. Rep.*, **21**, 1439–1447.
172. Zhang,J.J., Zhu,Y., Zhu,Y., Wu,J.L., Liang,W.B., Zhu,R., Xu,Z.K., Du,Q. and Miao,Y. (2012) Association of increased DNA methyltransferase expression with carcinogenesis and poor prognosis in pancreatic ductal adenocarcinoma. *Clin. Transl. Oncol.*, **14**, 116–124.
173. Jin,F., Dowdy,S.C., Xiong,Y.N., Eberhardt,N.L., Podratz,K.C. and Jiang,S.N. (2005) Up-regulation of DNA methyltransferase 3B expression in endometrial cancers. *Gynecol. Oncol.*, **96**, 531–538.
174. Yang,L., Hou,J., Cui,X.H., Suo,L.N. and Lv,Y.W. (2017) RG108 induces the apoptosis of endometrial cancer Ishikawa cell lines by inhibiting the expression of DNMT3B and demethylation of HMLH1. *Eur. Rev. Med. Pharmaco*, **21**, 5056–5064.
175. Lin,R.K., Wu,C.Y., Chang,J.W., Juan,L.J., Hsu,H.S., Chen,C.Y., Lu,Y.Y., Tang,Y.A., Yang,Y.C., Yang,P.C. *et al.* (2010) Dysregulation of p53/Sp1 Control Leads to DNA Methyltransferase-1 Overexpression in Lung Cancer. *Cancer Res.*, **70**, 5807–5817.

176. He,S.S., Wang,F., Yang,L.J., Guo,C.Y., Wan,R., Ke,A.W., Xu,L., Hu,G.Y., Xu,X.F., Shen,J. *et al.* (2011) Expression of DNMT1 and DNMT3a are regulated by GLI1 in human pancreatic cancer. *PLoS One*, **6**, e27684.
177. Yang,L., Xie,G., Fan,Q. and Xie,J. (2010) Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene*, **29**, 469–481.
178. Palakurthy,R.K., Wajapeyee,N., Santra,M.K., Gazin,C., Lin,L., Gobeil,S. and Green,M.R. (2009) Epigenetic silencing of the RASSF1A tumor suppressor gene through HOXB3-Mediated induction of DNMT3B expression. *Mol. Cell*, **36**, 219–230.
179. Deivendran,S., Marzook,H., Santhoshkumar,T.R., Kumar,R. and Pillai,M.R. (2017) Metastasis-associated protein 1 is an upstream regulator of DNMT3a and stimulator of insulin-growth factor binding protein-3 in breast cancer. *Sci. Rep.*, **7**, 44225.
180. Minarovits,J., Demcsak,A., Banati,F. and Niller,H.H. (2016) Epigenetic dysregulation in virus-associated neoplasms. *Adv. Exp. Med. Biol.*, **879**, 71–90.
181. Saito,Y., Kanai,Y., Sakamoto,M., Saito,H., Ishii,H. and Hirohashi,S. (2001) Expression of mRNA for DNA methyltransferases and methyl-CpG-binding proteins and DNA methylation status on CpG islands and pericentromeric satellite regions during human hepatocarcinogenesis. *Hepatology*, **33**, 561–568.
182. Jones,P.A. and Baylin,S.B. (2007) The epigenomics of cancer. *Cell*, **128**, 683–692.
183. Huang,C.R. and Lo,S.J. (2014) Hepatitis D virus infection, replication and cross-talk with the hepatitis B virus. *World J. Gastroentero*, **20**, 14589–14597.
184. Benegiamo,G., Vinciguerra,M., Guarnieri,V., Niro,G.A., Andriulli,A. and Pazienza,V. (2013) Hepatitis delta virus induces specific DNA methylation processes in Huh-7 liver cancer cells. *FEBS Lett.*, **587**, 1424–1428.
185. Jeong,S.W., Jang,J.Y. and Chung,R.T. (2012) Hepatitis C virus and hepatocarcinogenesis. *Clin. Mol. Hepatol.*, **18**, 347–356.
186. Hlady,R.A., Tiedemann,R.L., Puszyk,W., Zendejas,I., Roberts,L.R., Choi,J.H., Liu,C. and Robertson,K.D. (2014) Epigenetic signatures of alcohol abuse and hepatitis infection during human hepatocarcinogenesis. *Oncotarget*, **5**, 9425–9443.
187. Arora,P., Kim,E.O., Jung,J.K. and Jang,K.L. (2008) Hepatitis C virus core protein downregulates E-cadherin expression via activation of DNA methyltransferase 1 and 3b. *Cancer Lett.*, **261**, 244–252.
188. Shen,H., Wang,L., Spitz,M.R., Hong,W.K., Mao,L. and Wei,Q. (2002) A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. *Cancer Res.*, **62**, 4992–4995.
189. Jones,J.S., Amos,C.I., Pande,M., Gu,X., Chen,J., Campos,I.M., Wei,Q., Rodriguez-Bigas,M., Lynch,P.M. and Frazier,M.L. (2006) DNMT3b polymorphism and hereditary nonpolyposis colorectal cancer age of onset. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 886–891.
190. Coppede,F., Ricciardi,R., Denaro,M., De Rosa,A., Provenzano,C., Bartoccioni,E., Baggiani,A., Lucchi,M., Mussi,A. and Migliore,L. (2013) Association of the DNMT3B-579G >T polymorphism with risk of thymomas in patients with myasthenia gravis. *PLoS One*, **8**, e80846.
191. Chang,K.P., Hao,S.P., Liu,C.T., Cheng,M.H., Chang,Y.L., Lee,Y.S., Wang,T.H. and Tsai,C.N. (2007) Promoter polymorphisms of DNMT3B and the risk of head and neck squamous cell carcinoma in Taiwan: a case-control study. *Oral Oncol.*, **43**, 345–351.
192. Liu,H., Jiao,Y.F., Guan,Y.N., Lao,Y.B., Zhao,C.C. and Fan,H. (2012) The DNMT3B-579 G >T promoter polymorphism and risk of lung cancer. *Exp. Ther. Med.*, **3**, 525–529.
193. Fan,H., Liu,D.S., Qiu,X.M., Qiao,F.C., Wu,Q.X., Su,X.W., Zhang,F., Song,Y.W., Zhao,Z.J. and Xie,W. (2010) A functional polymorphism in the DNA methyltransferase-3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma. *BMC Med.*, **8**, https://doi.org/10.1186/1741-7015-8-12.
194. Ostler,K., Davis,E., Payne,S., Gosalia,B., Exposito-Cespedes,J., Le Beau,M.M. and Godley,L.A. (2007) Cancer cells express aberrant DNMT3B transcripts encoding truncated proteins. *Oncogene*, **26**, 5553–5563.
195. Gopalakrishnan,S., Van Emburgh,B.O., Shan,J.X., Su,Z., Fields,C.R., Vieweg,J., Hamazaki,T., Schwartz,P.H., Terada,N. and Robertson,K.D. (2009) A novel DNMT3B splice variant expressed in tumor and pluripotent cells modulates genomic DNA methylation patterns and displays altered DNA binding. *Mol. Cancer Res.*, **7**, 1622–1634.
196. Vasanthakumar,A., Lepore,J.B., Zegarek,M.H., Kocherginsky,M., Singh,M., Davis,E.M., Link,P.A., Anastasi,J., Le Beau,M.M., Karpf,A.R. *et al.* (2013) Dnmt3b is a haploinsufficient tumor suppressor gene in Myc-induced lymphomagenesis. *Blood*, **121**, 2059–2063.
197. Lopusna,K., Nowialis,P., Opavska,J., Abraham,A., Riva,A., Haney,S.L. and Opavsky,R. (2021) Decreases in different Dnmt3b activities drive distinct development of hematologic malignancies in mice. *J. Biol. Chem.*, **296**, 100285.
198. Wang,L., Wang,J., Sun,S.Y., Rodriguez,M., Yue,P., Jang,S.J. and Mao,L. (2006) A novel DNMT3B subfamily, Delta DNMT3B, is the predominant form of DNMT3B in non-small cell lung cancer. *Int. J. Oncol.*, **29**, 201–207.
199. Wang,J., Bhutani,M., Pathak,A.K., Lang,W., Ren,H., Jelinek,J., He,R., Shen,L., Issa,J.P. and Mao,L. (2007) Delta DNMT3B variants regulate DNA methylation in a promoter-specific manner. *Cancer Res.*, **67**, 10647–10652.
200. Iguchi,E., Takai,A., Takeda,H., Kumagai,K., Arasawa,S., Eso,Y., Shimizu,T., Ueda,Y., Marusawa,H. and Seno,H. (2020) DNA methyltransferase 3B plays a protective role against hepatocarcinogenesis caused by chronic inflammation via maintaining mitochondrial homeostasis. *Sci. Rep.*, **10**, 21268.
201. Jeltsch,A. and Jurkowska,R.Z. (2016) Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. *Nucleic Acids Res.*, **44**, 8556–8575.
202. Hervouet,E., Vallette,F.M. and Cartron,P.F. (2009) Dnmt3/transcription factor interactions as crucial players in targeted DNA methylation. *Epigenetics*, **4**, 487–499.
203. Estève,P.O., Chin,H.G. and Pradhan,S. (2005) Human maintenance DNA (cytosine-5)-methyltransferase and p53 modulate expression of p53-repressed promoters. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 1000–1005.
204. Brenner,C., Deplus,R., Didelot,C., Loriot,A., Vire,E., De Smet,C., Gutierrez,A., Danovi,D., Bernard,D., Boon,T. *et al.* (2005) Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J.*, **24**, 336–346.
205. Suzuki,M., Yamada,T., Kihara-Negishi,F., Sakurai,T., Hara,E., Tenen,D.G., Hozumi,N. and Oikawa,T. (2006) Site-specific DNA methylation by a complex of PU.1 and Dnmt3a/b. *Oncogene*, **25**, 2477–2488.
206. Lee,G.E. and Muller,M.T. (2006) DNMT3a interacts with Daxx to co-repress gene expression in the absence of DNA methylation. *Cancer Res.*, **66**, 5.
207. Di Croce,L., Raker,V.A., Corsaro,M., Fazi,F., Fanelli,M., Faretta,M., Fuks,F., Lo Coco,F., Kouzarides,T., Nervi,C. *et al.* (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science*, **295**, 1079–1082.
208. Cheray,M., Pacaud,R., Nadaradjane,A., Oliver,L., Vallette,F.M. and Cartron,P.F. (2016) Specific inhibition of DNMT3A/ISGF3γ interaction increases the temozolomide efficiency to reduce tumor growth. *Theranostics*, **6**, 1988–1999.
209. Fang,M., Ou,J., Hutchinson,L. and Green,M.R. (2014) The BRAF oncoprotein functions through the transcriptional repressor MAFG to mediate the CpG Island Methylator phenotype. *Mol. Cell*, **55**, 904–915.
210. Serra,R.W., Fang,M., Park,S.M., Hutchinson,L. and Green,M.R. (2014) A KRAS-directed transcriptional silencing pathway that mediates the CpG island methylator phenotype. *Elife*, **3**, e02313.
211. Robertson,K.D., Ait-Si-Ali,S., Yokochi,T., Wade,P.A., Jones,P.L. and Wolffe,A.P. (2000) DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.*, **25**, 338–342.
212. Pradhan,S. and Kim,G.-D. (2002) The retinoblastoma gene product interacts with maintenance human DNA (cytosine-5) methyltransferase and modulates its activity. *EMBO J.*, **21**, 779–788.

213. Butler,J.S., Lee,J.H. and Skalnik,D.G. (2008) CFP1 interacts with DNMT1 independently of association with the Setd1 Histone H3K4 methyltransferase complexes. *DNA Cell Biol.*, **27**, 533–543.
214. Cheray,M., Nadaradjane,A., Bonnet,P., Routier,S., Vallette,F.M. and Cartron,P.F. (2014) Specific inhibition of DNMT1/CFP1 reduces cancer phenotypes and enhances chemotherapy effectiveness. *Epigenomics*, **6**, 267–275.
215. Kar,S., Deb,M., Sengupta,D., Shilpi,A., Parbin,S., Torrisani,J., Pradhan,S. and Patra,S. (2012) An insight into the various regulatory mechanisms modulating human DNA methyltransferase 1 stability and function. *Epigenetics*, **7**, 994–1007.
216. Chuang,L.S., Ian,H.I., Koh,T.W., Ng,H.H., Xu,G. and Li,B.F. (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science*, **277**, 1996–2000.
217. Jimenji,T., Matsumura,R., Kori,S. and Arita,K. (2019) Structure of PCNA in complex with DNMT1 PIP box reveals the basis for the molecular mechanism of the interaction. *Biochem. Biophys. Res. Commun.*, **516**, 578–583.
218. Robertson,K.D. (2001) DNA methylation, methyltransferases, and cancer. *Oncogene*, **20**, 3139–3155.
219. Pacaud,R., Brocard,E., Lalier,L., Hervouet,E., Vallette,F.M. and Cartron,P.F. (2014) The DNMT1/PCNA/UHRF1 disruption induces tumorigenesis characterized by similar genetic and epigenetic signatures. *Sci. Rep.*, **4**, 4230.
220. Hervouet,E., Lalier,L., Debien,E., Cheray,M., Geairon,A., Rogniaux,H., Loussouarn,D., Martin,S.A., Vallette,F.M. and Cartron,P.F. (2010) Disruption of Dnmt1/PCNA/UHRF1 interactions promotes tumorigenesis from human and mice glial cells. *PLoS One*, **5**, e11333.
221. Wang,Y.A., Kamarova,Y., Shen,K.C., Jiang,Z., Hahn,M.J., Wang,Y. and Brooks,S.C. (2005) DNA methyltransferase-3a interacts with p53 and represses p53-mediated gene expression. *Cancer Biol. Ther.*, **4**, 1138–1143.
222. Fuks,F., Burgers,W.A., Godin,N., Kasai,M. and Kouzarides,T. (2001) Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. *EMBO J.*, **20**, 2536–2544.
223. Ma,P., de Waal,E., Weaver,J.R., Bartolomei,M.S. and Schultz,R.M. (2015) A DNMT3A2-HDAC2 Complex Is Essential for Genomic Imprinting and Genome Integrity in Mouse Oocytes. *Cell Rep.*, **13**, 1552–1560.
224. Geiman,T.M., Sankpal,U.T., Robertson,A.K., Zhao,Y. and Robertson,K.D. (2004) DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. *Biochem. Biophys. Res. Commun.*, **318**, 544–555.
225. Belinsky,S.A., Klinge,D.M., Stidley,C.A., Issa,J.P., Herman,J.G., March,T.H. and Baylin,S.B. (2003) Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res.*, **63**, 7089–7093.
226. Espada,J., Ballestar,E., Fraga,M.F., Villar-Garea,A., Juarranz,A., Stockert,J.C., Robertson,K.D., Fuks,F. and Esteller,M. (2004) Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J. Biol. Chem.*, **279**, 37175–37184.
227. Cheray,M., Pacaud,R., Nadaradjane,A., Vallette,F.M. and Cartron,P.F. (2013) Specific inhibition of one DNMT1-including complex influences tumor initiation and progression. *Clin Epigenetics*, **5**, 9.
228. Hernández-Muñoz,I., Taghavi,P., Kuijl,C., Neefjes,J. and van Lohuizen,M. (2005) Association of BMI1 with polycomb bodies is dynamic and requires PRC2/EZH2 and the maintenance DNA methyltransferase DNMT1. *Mol. Cell. Biol.*, **25**, 11047–11058.
229. Viré,E., Brenner,C., Deplus,R., Blanchon,L., Fraga,M., Didelot,C., Morey,L., Van Eynde,A., Bernard,D., Vanderwinden,J.-M. *et al.* (2006) The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*, **439**, 871–874.
230. Koya,J., Kataoka,K., Sato,T., Bando,M., Kato,Y., Tsuruta-Kishino,T., Kobayashi,H., Narukawa,K., Miyoshi,H., Shirahige,K. *et al.* (2016) DNMT3A R882 mutants interact with polycomb proteins to block haematopoietic stem and leukaemic cell differentiation. *Nat. Commun.*, **7**, 10924.
231. Li,H., Rauch,T., Chen,Z.X., Szabó,P.E., Riggs,A.D. and Pfeifer,G.P. (2006) The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. *J. Biol. Chem.*, **281**, 19489–19500.
232. Wu,X., Gong,Y., Yue,J., Qiang,B., Yuan,J. and Peng,X. (2008) Cooperation between EZH2, NSPc1-mediated histone H2A ubiquitination and Dnmt1 in HOX gene silencing. *Nucleic Acids Res.*, **36**, 3590–3599.
233. Rountree,M.R., Bachman,K.E. and Baylin,S.B. (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.*, **25**, 269–277.
234. Lee,G.E., Kim,J.H., Taylor,M. and Muller,M.T. (2010) DNA methyltransferase 1-associated protein (DMAP1) is a co-repressor that stimulates DNA methylation globally and locally at sites of double strand break repair. *J. Biol. Chem.*, **285**, 37630–37640.
235. Doyon,Y., Selleck,W., Lane,W.S., Tan,S. and Côté,J. (2004) Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. *Mol. Cell. Biol.*, **24**, 1884–1896.
236. Wang,J., Hevi,S., Kurash,J.K., Lei,H., Gay,F., Bajko,J., Su,H., Sun,W., Chang,H., Xu,G. *et al.* (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat. Genet.*, **41**, 125–129.
237. Brenner,C., Luciani,J., Bizet,M., Ndlovu,M., Josseaux,E., Dedeurwaerder,S., Calonne,E., Putmans,P., Cartron,P.-F., Defrance,M. *et al.* (2016) The interplay between the lysine demethylase KDM1A and DNA methyltransferases in cancer cells is cell cycle dependent. *Oncotarget*, **7**, 58939–58952.
238. Park,M.T. and Lee,S.J. (2003) Cell cycle and cancer. *J. Biochem. Mol. Biol.*, **36**, 60–65.
239. Williams,G.H. and Stoeber,K. (2012) The cell cycle and cancer. *J. Pathol.*, **226**, 352–364.
240. Lehnertz,B., Ueda,Y., Derijck,A.A., Braunschweig,U., Perez-Burgos,L., Kubicek,S., Chen,T., Li,E., Jenuwein,T. and Peters,A.H. (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.*, **13**, 1192–1200.
241. Datta,J., Majumder,S., Bai,S., Ghoshal,K., Kutay,H., Smith,D.S., Crabb,J.W. and Jacob,S.T. (2005) Physical and functional interaction of DNA methyltransferase 3A with Mbd3 and Brg1 in mouse lymphosarcoma cells. *Cancer Res.*, **65**, 10891–10900.
242. Myant,K. and Stancheva,I. (2008) LSH cooperates with DNA methyltransferases to repress transcription. *Mol. Cell. Biol.*, **28**, 215–226.
243. Xi,S., Zhu,H., Xu,H., Schmidtmann,A., Geiman,T.M. and Muegge,K. (2007) Lsh controls Hox gene silencing during development. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 14366–14371.
244. Ehrlich,M. (2009) DNA hypomethylation in cancer cells. *Epigenomics*, **1**, 239–259.
245. Fan,T., Schmidtmann,A., Xi,S., Briones,V., Zhu,H., Suh,H.C., Gooya,J., Keller,J.R., Xu,H., Roayaei,J. *et al.* (2008) DNA hypomethylation caused by Lsh deletion promotes erythroleukemia development. *Epigenetics*, **3**, 134–142.
246. Hashimoto,H., Vertino,P.M. and Cheng,X. (2010) Molecular coupling of DNA methylation and histone methylation. *Epigenomics*, **2**, 657–669.
247. Cheng,X. (2014) Structural and functional coordination of DNA and histone methylation. *Cold Spring Harb. Perspect. Biol.*, **6**, a018747.
248. Duymich,C.E., Charlet,J., Yang,X., Jones,P.A. and Liang,G. (2016) DNMT3B isoforms without catalytic activity stimulate gene body methylation as accessory proteins in somatic cells. *Nat. Commun.*, **7**, 11453.
249. Masalmeh,R.H.A., Taglini,F., Rubio-Ramon,C., Musialik,K.I., Higham,J., Davidson-Smith,H., Kafetzopoulos,I., Pawlicka,K.P., Finan,H.M., Clark,R. *et al.* (2021) De novo DNA methyltransferase activity in colorectal cancer is directed towards H3K36me3 marked CpG islands. *Nat. Commun.*, **12**, 694.
250. Zhang,Y., Jurkowska,R., Soeroes,S., Rajavelu,A., Dhayalan,A., Bock,I., Rathert,P., Brandt,O., Reinhardt,R., Fischle,W. *et al.* (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Res.*, **38**, 4246–4253.
251. Li,B.Z., Huang,Z., Cui,Q.Y., Song,X.H., Du,L., Jeltsch,A., Chen,P., Li,G., Li,E. and Xu,G.L. (2011) Histone tails regulate DNA

methylation by allosterically activating de novo methyltransferase. *Cell Res.*, **21**, 1172–1181.
252. Hodges,E., Smith,A.D., Kendall,J., Xuan,Z., Ravi,K., Rooks,M., Zhang,M.Q., Ye,K., Bhattacharjee,A., Brizuela,L. *et al.* (2009) High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res.*, **19**, 1593–1605.
253. Meissner,A., Mikkelsen,T.S., Gu,H., Wernig,M., Hanna,J., Sivachenko,A., Zhang,X., Bernstein,B.E., Nusbaum,C., Jaffe,D.B. *et al.* (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature*, **454**, 766–770.
254. Petell,C.J., Alabdi,L., He,M., San Miguel,P., Rose,R. and Gowher,H. (2016) An epigenetic switch regulates de novo DNA methylation at a subset of pluripotency gene enhancers during embryonic stem cell differentiation. *Nucleic Acids Res.*, **44**, 7605–7617.
255. AlAbdi,L., Saha,D., He,M., Dar,M.S., Utturkar,S.M., Sudyanti,P.A., McCune,S., Spears,B.H., Breedlove,J.A., Lanman,N.A. *et al.* (2020) Oct4-mediated inhibition of Lsd1 activity promotes the active and primed state of pluripotency enhancers. *Cell Rep.*, **30**, 1478–1490.
256. Qin,W., Wolf,P., Liu,N., Link,S., Smets,M., La Mastra,F., Forné,I., Pichler,G., Hörl,D., Fellinger,K. *et al.* (2015) DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. *Cell Res.*, **25**, 911–929.
257. Nishiyama,A., Yamaguchi,L., Sharif,J., Johmura,Y., Kawamura,T., Nakanishi,K., Shimamura,S., Arita,K., Kodama,T., Ishikawa,F. *et al.* (2013) Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature*, **502**, 249–253.
258. Jin,B., Ernst,J., Tiedemann,R.L., Xu,H., Sureshchandra,S., Kellis,M., Dalton,S., Liu,C., Choi,J.-H. and Robertson,K.D. (2012) Linking DNA methyltransferases to epigenetic marks and nucleosome structure genome-wide in human tumor cells. *Cell Rep.*, **2**, 1411–1424.
259. Jinawath,A., Miyake,S., Yanagisawa,Y., Akiyama,Y. and Yuasa,Y. (2005) Transcriptional regulation of the human DNA methyltransferase 3A and 3B genes by Sp3 and Sp1 zinc finger proteins. *Biochem. J.*, **385**, 557–564.
260. Kishikawa,S., Murata,T., Kimura,H., Shiota,K. and Yokoyama,K.K. (2002) Regulation of transcription of the Dnmt1 gene by Sp1 and Sp3 zinc finger proteins. *Eur. J. Biochem.*, **269**, 2961–2970.
261. Szemes,M., Dallosso,A.R., Melegh,Z., Curry,T., Li,Y., Rivers,C., Uney,J., Mägdefrau,A.S., Schwiderski,K., Park,J.H. *et al.* (2013) Control of epigenetic states by WT1 via regulation of de novo DNA methyltransferase 3A. *Hum. Mol. Genet.*, **22**, 74–83.
262. Agrawal,K., Das,V., Vyas,P. and Hajdúch,M. (2018) Nucleosidic DNA demethylating epigenetic drugs - a comprehensive review from discovery to clinic. *Pharmacol. Ther.*, **188**, 45–79.
263. Gilmartin,A.G., Groy,A., Gore,E.R., Atkins,C., Long,E.R., Montoute,M.N., Wu,Z., Halsey,W., McNulty,D.E., Ennulat,D. *et al.* (2021) In vitro and in vivo induction of fetal hemoglobin with a reversible and selective DNMT1 inhibitor. *Haematologica*, **106**, 1979–1987.
264. Pappalardi,M.B., Keenan,K., Cockerill,M., Kellner,W.A., Stowell,A., Sherk,C., Wong,K., Pathuri,S., Briand,J., Steidel,M. *et al.* (2021) Discovery of a first-in-class reversible DNMT1-selective inhibitor with improved tolerability and efficacy in acute myeloid leukemia. *Nature Cancer*.**2**, 1002–1017.
265. Cartron,P.F., Blanquart,C., Hervouet,E., Gregoire,M. and Vallette,F.M. (2013) HDAC1-mSin3a-NCOR1, Dnmt3b-HDAC1-Egr1 and Dnmt1-PCNA-UHRF1-G9a regulate the NY-ESO1 gene expression. *Mol Oncol*, **7**, 452–463.
266. Zhang,Y., Jurkowska,R., Soeroes,S., Rajavelu,A., Dhayalan,A., Bock,I., Rathert,P., Brandt,O., Reinhardt,R., Fischle,W. *et al.* (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic Acids Res.*, **38**, 4246–4253.
267. Zhang,J., Yang,C., Wu,C., Cui,W. and Wang,L. (2020) DNA methyltransferases in cancer: biology, paradox, aberrations, and targeted therapy. *Cancers (Basel)*, **12**, 2123.
268. Hamidi,T., Singh,A.K. and Chen,T. (2015) Genetic alterations of DNA methylation machinery in human diseases. *Epigenomics*, **7**, 247–265.
269. Tang,Z.F., Kang,B.X., Li,C.W., Chen,T.X. and Zhang,Z.M. (2019) GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.*, **47**, W556–W560.
270. Puto,L.A., Benner,C. and Hunter,T. (2015) The DAXX co-repressor is directly recruited to active regulatory elements genome-wide to regulate autophagy programs in a model of human prostate cancer. *Oncoscience*, **2**, 362–372.
271. Pang,Y., Liu,J., Li,X., Xiao,G., Wang,H., Yang,G., Li,Y., Tang,S.C., Qin,S., Du,N. *et al.* (2018) MYC and DNMT3A-mediated DNA methylation represses microRNA-200b in triple negative breast cancer. *J. Cell. Mol. Med.*, **22**, 6262–6274.
272. Lee,H., Zhang,P., Herrmann,A., Yang,C., Xin,H., Wang,Z., Hoon,D.S., Forman,S.J., Jove,R., Riggs,A.D. *et al.* (2012) Acetylated STAT3 is crucial for methylation of tumor-suppressor gene promoters and inhibition by resveratrol results in demethylation. *Proc. Natl. Acad. Sci. U.S.A.*, **109**, 7765–7769.
273. Lee,C.F., Ou,D.S., Lee,S.B., Chang,L.H., Lin,R.K., Li,Y.S., Upadhyay,A.K., Cheng,X., Wang,Y.C., Hsu,H.S. *et al.* (2010) hNaa10p contributes to tumorigenesis by facilitating DNMT1-mediated tumor suppressor gene silencing. *J. Clin. Invest.*, **120**, 2920–2930.
274. Fuks,F., Burgers,W.A., Brehm,A., Hughes-Davies,L. and Kouzarides,T. (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat. Genet.*, **24**, 88–91.
275. Cheng,J., Yang,H., Fang,J., Ma,L., Gong,R., Wang,P., Li,Z. and Xu,Y. (2015) Molecular mechanism for USP7-mediated DNMT1 stabilization by acetylation. *Nat. Commun.*, **6**, 7023.
276. Li,J., Wang,R., Jin,J., Han,M., Chen,Z., Gao,Y., Hu,X., Zhu,H., Gao,H., Lu,K. *et al.* (2020) USP7 negatively controls global DNA methylation by attenuating ubiquitinated histone-dependent DNMT1 recruitment. *Cell Discovery*, **6**, 58.
277. Hu,Y., Wu,F., Liu,Y., Zhao,Q. and Tang,H. (2019) DNMT1 recruited by EZH2-mediated silencing of miR-484 contributes to the malignancy of cervical cancer cells through MMP14 and HNF1A. *Clin. Epigenet.*, **11**, 186.
278. Pangeni,R.P., Yang,L., Zhang,K., Wang,J., Li,W., Guo,C., Yun,X., Sun,T., Wang,J. and Raz,D.J. (2020) G9a regulates tumorigenicity and stemness through genome-wide DNA methylation reprogramming in non-small cell lung cancer. *Clin. Epigenet.*, **12**, 88.
279. Estève,P.-O., Chin,H.G., Smallwood,A., Feehery,G.R., Gangisetty,O., Karpf,A.R., Carey,M.F. and Pradhan,S. (2006) Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes Dev.*, **20**, 3089–3103.
280. Dong,C., Wu,Y., Wang,Y., Wang,C., Kang,T., Rychahou,P.G., Chi,Y.I., Evers,B.M. and Zhou,B.P. (2013) Interaction with Suv39H1 is critical for Snail-mediated E-cadherin repression in breast cancer. *Oncogene*, **32**, 1351–1362.