Published in final edited form as:

Adv Exp Med Biol. 2013 ; 754: 3–29. doi:10.1007/978-1-4419-9967-2_1.

DNA Methyltransferases (DNMTs), DNA Damage Repair, and Cancer

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Abstract

The maintenance DNA methyltransferase (DNMT) 1 and the *de novo* methyltransferases DNMT3A and DNMT3B are all essential for mammalian development. DNA methylation, catalyzed by the DNMTs, plays an important role in maintaining genome stability. Aberrant expression of DNMTs and disruption of DNA methylation patterns are closely associated with many forms of cancer, although the exact mechanisms underlying this link remain elusive. DNA damage repair systems have evolved to act as a genome-wide surveillance mechanism to maintain chromosome integrity by recognizing & repairing both exogenous and endogenous DNA insults. Impairment of these systems gives rise to mutations and directly contributes to tumorigenesis. Evidence is mounting for a direct link between DNMTs, DNA methylation, and DNA damage repair systems, which provide new insight into the development of cancer. Like tumor suppressor genes (TSGs), an array of DNA repair genes frequently sustain promoter hypermethylation in a variety of tumors. In addition, DNMT1, but not the DNMT3's, appear to function coordinately with DNA damage repair pathways to protect cells from sustaining mutagenic events, which is very likely through a DNA methylation-independent mechanism. This chapter is focused on reviewing the links between DNA methylation and the DNA damage response.

Keywords

DNA methyltransferase; DNA methylation; genome stability; DNA damage repair

Introduction

DNA methyltransferases (DNMTs), responsible for the transfer of a methyl group from the universal methyl donor, *S*-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA, are essential for mammalian development¹. There are four members of the DNMT family, including DNMT1, DNMT3A, DNMT3B and DNMT3L. DNMT3L, unlike the other DNMTs, does not possess any inherent enzymatic activity². The other three family members are active on DNA. *DNMT1* encodes the maintenance methyltransferase and *DNMT3A/DNMT3B* encode the *de novo* methyltransferases^{3–4}, required to establish and maintain genomic methylation. While this maintenance vs *de novo* division has been convenient, there is clear evidence for functional overlap between the maintenance and the *de novo* methyltransferases^{5–6}. Gene knockout analysis in mice has shown that *Dnmt1* and *Dnmt3a/Dnmt3b* genes are all essential for viability. *Dnmt1*-inactivation leads to very early lethality at embryonic day (E) 9.5, shortly after gastrulation^{7–9}, whereas *Dnmt3b* knockout induces embryo death at E14.5–18.5, due to multiple developmental defects including

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growth impairment and rostral neural tube defects^{3, 8-9}. *Dnmt3a*^{-/-} mice become runted and die at about 4 weeks of age, although they appear to be relatively normal at birth³.

DNMTs play an important role in genomic integrity, disruption of which may result in chromosome instability and tumor progression. It is well established that DNMTs are required for transcriptional silencing of a number of sequence classes, including imprinted genes, genes on the inactive X chromosome and transposable elements^{1, 10}, and silencing of these sequences is essential for maintaining chromosome stability. Much compelling evidence has come from targeted deletion experiments showing that all three DNA methyltransferases are involved in stabilization of the genome, particularly repetitive sequences^{3, 11-12}. For example, either single knockout of *Dnmt1* or double knockout of *Dnmt3a* and *Dnmt3b*, enhances telomere recombination¹¹. DNMT3B is specifically required for stabilization of pericentromeric satellite repeats. DNMT3B deficiency results in expansion and rearrangements of pericentromeric repeats^{3, 12}. ICF (Immunodeficiency, Centromere instability and Facial anomalies) syndrome is the only human genetic disorder known to involve biallelic mutations in *DNMT3B*. It is characterized by chromosomal instability arising due to destabilization of pericentromeric repeats, particular those at juxtacentromeric regions of chromosomes 1, 9 and 16^{3, 12}. Of note, cells null for *DNMT1* or with hypomorphic mutations in *DNMT1* that partially reduce its levels to 30% of WT DNMT1, display significantly greater microsatellite instability¹³⁻¹⁷, a greater frequency of chromosomal translocations¹⁸ and much higher sensitivity to genotoxic agents¹⁷, which may promote the development of cancer.

The DNA damage response (DDR) is a genome-wide surveillance system that protects cells from potentially mutagenic DNA insults derived from either endogenous or exogenous sources. The DDR usually functions through the coordinated actions of DNA repair and checkpoint systems to promote DNA damage repair before replication or to activate cell death pathways if excessive damage exists¹⁹. Like the cellular DNA methylation machinery, an intact DDR is crucial for preventing cancer. Evidence is mounting to support a link between the DNA methylation and DNA damage repair systems, as first suggested by promoter hypermethylation & silencing of DNA repair genes in multiple types of cancer²⁰. More importantly, DNMT1 may be directly involved in DNA damage repair in a DNA methylation-independent manner^{14, 17, 21-23}. Strong support for this latter notion comes from recent observations that DNMT1 is rapidly and transiently recruited to regions of DNA double strand breaks (DSBs) via its interaction with proliferating cell nuclear antigen (PCNA)^{21, 24}, as well the PCNA-like DNA damage sliding clamp component RAD9 (of the 9-1-1 complex)²¹. In this chapter, we examine and outline the links between DNMTs and DNA repair systems and discuss the possible mechanisms of how they are orchestrated, with a focus on cancer.

1. Epigenetic Silencing of DNA Repair Pathways through Aberrant Promoter Hypermethylation

DNA repair systems have evolved to maintain genomic integrity by countering threats posed by DNA lesions¹⁹. Deficiency in the DNA repair pathways may leave these lesions unrepaired or cause them to be repaired incorrectly, eventually leading to genome instability or mutations that contribute directly to a large array of human diseases including cancer. Carcinogenesis is believed to originate from and be driven by the acquisition of abnormal genetic and/or epigenetic changes. Aberrant DNA hypermethylation, when it occurs at promoter CpG islands (CGIs), leads to potent and heritable transcriptional silencing that inactivates key cellular pathways much like genetic changes (e.g. mutation/deletion) do. In addition to genetic mutations, promoter hypermethylation in DNA repair genes is closely linked to a variety of human tumor types including colorectal, breast, lung cancers and

glioma²⁰ (Table 1), suggesting that epigenetic silencing of DNA repair pathways is an important contributor to the development of cancer.

1.1 Epigenetic Inactivation of the DNA Mismatch Repair (MMR) Pathway

MMR is a genome-surveillance system to maintain genomic integrity through recognizing and correcting mismatched nucleotides arising during DNA replication, homologous recombination (HR), or other forms of DNA damage. Impairment of this system gives rise to microsatellite instability (MSI)^{25–26}, which has now been recognized as a hallmark of MMR gene-deficient cancers. Microsatellite loci, widely dispersed in the genome, are repetitive sequences consisting of short runs of nucleotides, typically one to four bases in length. Repetitive regions may give rise to the formation of secondary structures, which are subject to expansion or contraction. The secondary structures, if incorrectly resolved, lead to slippage of DNA polymerases along repetitive sequences during replication. Microsatellites are particularly susceptible to length change mutations during replication and transcription, resulting in frameshift mutations if they are located within a gene^{25–26}. MMR deals with these changes to maintain microsatellite stability. MMR comprises the MutS complex and the MutL complex. MutS recognizes the mismatched base, while MutL recruits repair enzymes to damage sites via its binding with MutS²⁷. There are two main MutS complexes in humans, MutS α and MutS β . MutS α , consisting of the MutS homologue 2 (MSH2) protein bound to MSH6, recognizes single-base mismatches or small insertion/deletion loops (indels), whereas MutS β , consisting of MSH2 and MSH3, repairs only indels²⁸. The main complex for MutL in humans is MutL α , consisting of a heterodimer of MLH1 and PMS2²⁶. Mutations in or epigenetic silencing of MMR genes like *MLH1* and *MSH2* is closely associated with a variety of human cancers such as hereditary non-polyposis colon cancer (HNPCC), sporadic colon cancer, and ovarian cancer²⁹.

MLH1 plays a central role in coordinating various steps in MMR via interacting with other MMR proteins and modulating their activities. Hypermethylation of the *MLH1* promoter is observed in a variety of cancers including oral squamous cell carcinoma³⁰, gastric cancer^{31–32}, non-small cell lung cancer (NSCLC)³³, ovarian cancer³⁴, acute myeloid leukemia^{35–37}, head and neck squamous cell carcinoma (HNSCC)³⁸, HNPCC^{39–41}, and particularly in colorectal cancer (CRC)^{42–45} (Table 1). The reduced MLH1 protein expression is correlated with high-level methylation detected in human CRC samples, whereas samples with low-level methylation display expression levels similar to those observed in methylation-negative samples⁴⁶, strongly suggesting that the *MLH1* gene is inactivated via promoter hypermethylation in a dose-dependent manner. Nonetheless, it is not clear whether a moderate degree of methylation affects MLH1 gene expression or not. On the basis of observations made in germline cells, it has long been believed that *MLH1* promoter methylation involves only one allele of maternal origin. However, more recent findings demonstrate that there is biallelic involvement of *MLH1* promoter hypermethylation in many cancers⁴⁶. The causal link between MSI and epigenetic inactivation of *MLH1* is further highlighted by the observation that 90% of MSI+ HNPCC have *MLH1* hypermethylation, while 95% of MSI- samples do not²⁰.

MSH2 is also hypermethylated in multiple tumor types, including gastric cancer³¹, NSCLC³³, ovarian cancer⁴⁷, sporadic CRC⁴⁸, and HNPCC⁴⁹ (Table 1). Interestingly, promoter methylation of *MSH2* in HNPCC occurs primarily in patients with germline mutations in *MSH2* rather than in germline mutation-negative cases⁴⁹. Seventy percent of patients with *MSH2* methylation also present germline mutations in this gene, clearly indicating that methylation is the second inactivating hit in these tumors⁴⁹. DNA hypermethylation can be caused by transcription across a CpG island within a promoter region. Recent studies have revealed that deletions of the last exons of the *EpCAM* gene, located immediately upstream of *MSH2*, give rise to somatic hypermethylation of the *MSH2*

promoter⁵⁰. Deletions at the most 3'-end of the *EpCAM* gene result in loss of its polyadenylation signal, which abolishes transcription termination. Transcription of *EpCAM* then continues downstream into the *MSH2* promoter and induces promoter hypermethylation of *MSH2*. DNA methylation triggered by transcriptional read-through of a neighboring gene, in either sense or antisense, may represent a general mutational mechanism that promotes aberrant epigenetic changes. Like *MLH2*, other MutS homologues, including *MSH3* and *MSH6*, are also inactivated by hypermethylation in tumors such as breast⁵¹ and gastric cancers³¹ (Table 1).

1.2 Epigenetic Inactivation of the Base Excision Repair (BER) and Nucleotide Excision Repair (NER) Pathway

The specific pairing of DNA bases in the genome is constantly challenged by endogenous metabolic byproducts and environmental insults. BER is responsible for the removal of damaged DNA bases and their backbones to prevent mutations that could give rise to cancer^{19, 52}. In BER, abnormal DNA bases are recognized and removed by specific glycosylases, followed by recruitment of other enzymes including nuclease, polymerase and ligase proteins, to complete the repair process via excising the remaining sugar fragments and reinstalling an intact correctly based-paired nucleotide¹⁹.

Either thymine DNA glycosylase (TDG) or methyl-CpG binding domain 4 (MBD4), mediate a specific BER pathway for the correction of G/T mismatches arising due to 5-methylcytosine deamination leading to C to T transitions. DNA hypermethylation-mediated silencing of *TDG* and *MBD4* may contribute to the frequent genomic instability that occurs in cancer cells⁵³ (Table 1). *TDG* promoter hypermethylation negatively correlates with its expression. TDG down-regulation leads to less efficient DNA repair activity in response to hydrogen peroxide-induced DNA damage. Ectopic expression of TDG, however, functionally compensates for lower repair activities of damaged DNA in the KAS-6/1 myeloma cell line with extensive endogenous *TDG* gene hypermethylation⁵³. *MBD4*, like *TDG*, is also subject to promoter hypermethylation and gene silencing in tumors like sporadic CRC and ovarian cancer⁵⁴. Another DNA glycosylase, OGG1, which mediates removal of 8-oxoguanine induced by oxidative damage, is also subject to inactivation via promoter methylation in cancer cells⁵⁵ (Table 1).

Of all the repair systems, NER recognizes the most varied types of DNA lesions, contending with the diverse class of helix-distorting damage that interferes with base pairing and obstructs replication and transcription. In NER, there exist two sub-pathways that differ in the mechanism of lesion recognition: global genome NER (GG-NER) that surveys the entire genome for distortions, and transcription-coupled repair (TCR), which targets damage that blocks elongating RNA polymerases^{19, 56}. NER, therefore, plays a particularly important role in preventing mutations. Thus far, three syndromes, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy (TTD), are closely associated with NER defects⁵⁶. Of these, patients with xeroderma pigmentosum, attributable to mutations in one of seven xeroderma pigmentosum (XP) group genes (*XPA*–*XPG*), show a dramatically increased incidence of UV light-induced skin cancer^{19, 56}.

It was reported recently that the *XPC* promoter is epigenetically inactivated in bladder cancer⁵⁷ (Table 1). *XPC* promoter methylation is significantly elevated in cancerous bladder compared to normal tissue, leading to reduced mRNA levels in the tumor⁵⁷. Epigenetic defects in the *XPC* gene may also influence malignant behavior and prognosis. ERCC1 is a crucial protein in the NER pathway primarily involved in the repair of platinum-DNA adducts. Aberrant CpG island methylation in the *ERCC1* promoter region has been observed in human glioma cell lines and primary tumors, which is associated with cisplatin chemosensitivity⁵⁸. In a rat lung cancer model, however, *ERCC1* methylation is detected in

only a very small proportion of samples⁵⁹. Deficiency in XRCC1, a scaffolding protein for BER and single-strand break repair (SSBR), is associated with enhanced risk of lung cancer⁶⁰. *XRCC1* is subject to aberrant promoter methylation in human gastric cancer tissues⁶¹. In lung cancer, infiltrating carcinomas exhibit statistically higher levels of methylation at the *XRCC1* promoter compared to normal, hyperplastic, and squamous metaplastic tissues⁵⁹. RAD23B, a key component for damage recognition in NER, is also hypermethylated in multiple myeloma⁶².

1.3 Epigenetic Inactivation of HR and Non-Homologous End-Joining (NHEJ) DNA Repair Pathway Components

HR not only provides an important mechanism to repair several types of DNA lesions that pose a threat to genome integrity, including DNA DSBs, DNA damage encountered during DNA replication and DNA interstrand cross links (ICLs), but is also required to restart stalled replication forks during the late S and G2 phases of the cell cycle^{63–64}. HR promotes precise repair of DNA damage using the intact sister chromatid as a template. Deficiency of HR leads to more error-prone repair, which is associated with mutagenesis and predisposition to cancer⁶³.

The *BRCA1* and *BRCA2* genes are both essential for HR-mediated DNA repair. *BRCA1* appears to act as a signal integrator that links DNA damage sensors with response mechanisms. *BRCA2*, however, is more directly involved in homology-directed DSB repair, as it mediates formation of a RAD51-DNA nucleoprotein filament that catalyzes strand invasion during HR. *BRCA1* and *BRCA2* are frequently mutated in hereditary breast and ovarian cancers, but seldom in sporadic cases of these tumor types. Epigenetic inactivation of *BRCA1* via promoter hypermethylation, however, plays an important role in tumorigenesis in a wide array of cancers including breast^{65–66}, ovarian⁶⁷, gastric⁶⁸, bladder⁶⁹ and non-small cell lung cancers⁷⁰, both hereditary⁷¹ and sporadic forms^{20, 39} (Table 1). It is believed that epigenetic silencing of *BRCA1* creates a new mutator pathway that generates mutations and gross chromosomal rearrangements via p53 signaling. This idea is supported by several observations including one demonstrating that p53 inactivation rescues the impact of *BRCA1* deficiency on cell survival^{20, 72}. Although much less frequently than *BRCA1*, *BRCA2* also acquires promoter region hypermethylation that is closely associated with its reduced expression in breast cancer⁵¹ and NSCLC⁷⁰ (Table 1).

The primary function of the Fanconi anemia (FA) pathway is to repair inter-strand DNA cross-links, which promotes HR via coordinating other DNA damage-responsive events to stabilize stalled replication forks, to convey signals to DNA checkpoint pathways, and to facilitate recovery of replication forks⁷³. FA is a genomic instability syndrome characterized by bone marrow failure, developmental abnormalities, and increased cancer incidence, which is caused by mutations in one of thirteen distinct genes (*FANCA*, *FANCB*, *FANCC*, *FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, and *FANCN*)⁷³. Eight of them (*FANCA*, *B*, *C*, *E*, *F*, *G*, *L* and *M*) form the FA core complex. This group of genes contain a high GC content and CpG islands at their promoter regions, making them potential targets for aberrant hypermethylation-mediated silencing⁷⁴. This idea has received support from observations that *FANCC*, *FANCF* and *FANCL* acquire promoter methylation during human carcinogenesis^{39, 75}. Of these, *FANCF* displays hypermethylation the most frequently, occurring in 14% to 28% of different cancers including NSCLC⁷⁶, HNSCC⁷⁶, cervical⁷⁷, and ovarian^{39, 78} (Table 1).

Unlike HR, which performs error-free repair, NHEJ simply restores DNA integrity by joining the two DNA ends. This type of repair is error-prone and frequently results in the loss or addition of several nucleotides at the break site. Despite its mutagenic consequences, NHEJ is the major DSB repair pathway in mammalian cells. Defects in NHEJ lead to

chromosomal translocations and genomic instability. In NHEJ, DSBs are detected by the KU70/KU80 heterodimer; the KU complex then activates the protein kinase DNA-PKcs (DNA-dependent protein kinase catalytic subunit), leading to recruitment and activation of end-processing enzymes, polymerases and finally ligation of the breaks by the XRCC4/DNA ligase IV complex. In the NHEJ pathway, only the *XRCC5* gene, encoding the KU80 protein, has been reported to be inactivated via epigenetic mechanisms⁷⁰ (Table 1). Low expression of *XRCC5* in squamous cell carcinoma and NSCLC is significantly associated with promoter region hypermethylation. Treatment of NSCLC cells with the DNA demethylating agent 5-aza-2'-deoxycytidine (5-aza-CdR), however, does not result in increased KU80 expression⁷⁰. Thus, the underlying mechanisms promoting and maintaining *XRCC5* silencing await further investigation, particularly in more samples and more types of cancer.

1.4 Epigenetic Silencing of O⁶-methylguanine-DNA Methyltransferase (MGMT)

O⁶-methylguanine, which arises due to alkylation reactions, pairs with thymine rather than cytosine, resulting in G:C to A:T mutations during DNA replication. MGMT, also known as O⁶-alkylguanine-DNA alkyltransferase (AGT), repairs DNA damage by transferring the methyl groups on the O⁶ position of guanine to an active site cysteine residue to protect cells from sustaining mutagenic events, which has been demonstrated by gain- or loss-of function experiments *in vitro* and *in vivo*⁷⁹. The MGMT protein is unique among DNA-repair components because it acts alone to remove DNA adducts. Although MGMT is ubiquitously expressed in normal human tissues, mean enzymatic activity in malignant tissues is usually higher than in their normal counterparts. However, there is a variety of tumors such as glioma, CRC, NSCLC and HNSCC that lack MGMT expression^{20, 39} (Table 1). It has been well documented that MGMT deficiency often arises due to abnormal promoter methylation^{20, 39, 80}. For example, 29% of NSCLCs and 38% of CRCs display aberrant *MGMT* methylation, in which the presence of hypermethylation is highly associated with loss of MGMT protein⁸¹. *MGMT* is the most frequently methylated gene in central nervous system tumors. Epigenetic silencing of *MGMT* via promoter hypermethylation occurs in approximately 40% of primary glioblastomas and over 70% of secondary glioblastomas. It is also detected in 50% of the diffuse and anaplastic astrocytomas and approximately two thirds of oligodendrogial and mixed tumors⁸². These results, together with a causal relationship between DNA methylation of the *MGMT*CpG island and decreased transcription of the gene in cell culture-based studies, demonstrates that DNA methylation is an important mechanism for silencing the *MGMT* gene in human cancers.

Epigenetic silencing of *MGMT* may initiate an important mutator signaling cascade in human cancers since MGMT loss causes G:C to A:T transitions, which lead to downstream gene mutations. This proposal is strongly supported by an analysis of point mutations in *KRAS* and *p53*. *KRAS*, the most commonly altered oncogene in cancer, is an early key player in multiple signal pathways. Loss of MGMT is associated with increased *KRAS* mutations possessing G:C to A:T transitions in colon⁸³ and gastric cancer⁸⁴. *p53* is the most frequently mutated TSG (tumor suppressor gene) in human cancer, and the majority of known *p53* mutations are G:C to A:T transitions^{66, 85}. Epigenetic inactivation of *MGMT* may lead to G:C to A:T transition mutations in *p53*, which has been observed in several types of cancer including colorectal⁶⁶, liver⁸⁶, lung⁸⁷, esophageal squamous cell carcinomas⁸⁸ and glioma⁸⁹. Interestingly, *MGMT* promoter methylation is associated with improved disease chemo-sensitivity and prolonged survival time in patients treated with alkylating agent-based therapies⁹⁰. However, it is unclear whether the improved survival is specifically due to loss of MGMT expression or accompanying drug sensitivity.

1.5 Epigenetic Silencing of WRN

Werner syndrome (WS) is a rare autosomal recessive disease, characterized by premature onset of aging, genomic instability, and increased cancer incidence. WS is caused by null mutations at the *WRN* locus at 8p11.2–p12, which codes for a DNA helicase belonging to the RecQ family. Deficiency in WRN function causes defects in DNA replication and recombination, as well as DNA repair.

WRN is a 180-kd nuclear protein that has a unique interaction with its DNA substrates through its C terminal RQC domain during base-separation⁹¹. In addition to two C-terminal ATPase domains encoding for helicase activity, the WRN protein contains an N-terminal domain coding for exonuclease activity. Its helicase and exonuclease activities function in a coordinated manner, suggestive of roles in DNA repair, recombination, and replication. Recently, the WRN protein was also shown to be involved in telomere maintenance based on the discovery that its deficiency leads to accelerated telomere shortening in WS cells⁹². These multiple roles of the WRN protein highlight its importance in aging and cancer.

The evidence suggesting that WRN acts as a tumor suppressor gene are derived primarily from WS, which is characterized by the early onset development of a variety of cancers due to germline *WRN* mutation; somatic mutations in the *WRN* gene have not been reported. Epigenetic inactivation of *WRN* provides additional support for its TSG role in sporadic cancer. The *WRN* promoter undergoes hypermethylation in a wide array of tumors including colorectal, gastric, prostate, non-small cell lung and breast cancers^{93–94} (Table 1). Epigenetic silencing of *WRN* via methylation not only leads to the loss of protein and enzyme activity, but also to chromosomal instability. Furthermore, the above phenotype is reversed by DNA-demethylating agents. Most importantly, restoration of WRN expression induces its tumor-suppressor effects, such as inhibition of colony formation and tumor growth⁹³. Taken together, aberrant epigenetic silencing of *WRN*, a candidate TSG, may play an important role in human cancers. Interestingly, WRN was recently shown to be associated with promoter methylation of the *OCT4* gene⁹⁵, which encodes a crucial transcription factor for the maintenance of cell pluripotency. During differentiation of human pluripotent NCCIT embryonic carcinoma cells, WRN localizes to the *OCT4* promoter region with *de novo* DNA methyltransferase DNMT3B and promotes differentiation-dependent *OCT4* silencing and promoter methylation⁹⁵. Deficiency in WRN blocks DNMT3B recruitment to the promoter and leads to decreased promoter methylation of *OCT4*⁹⁵. Therefore, WRN may also contribute to the control of stem cell differentiation via epigenetic silencing of the key pluripotency transcription factor OCT4.

1.6 Epigenetic Inactivation of ATM/ATR Signaling

DNA damage signaling requires the coordinated action of a large array of molecules that can be categorized as DNA damage sensors, transducers, mediators, and effectors according to their functions. Upon damage of DNA, the MRE11–RAD50–NBS1 (MRN) sensor complex recognizes DSBs and the replication protein A (RPA) complex processes accumulated single stranded DNA (ssDNA). The transducer ATM and ATR kinases are recruited to and activated by DSBs and RPA-coated ssDNA, respectively. With the help of mediators (including 53BP1, MDC1, BRCA1, MCPH1 and PTIP in ATM signaling, and TopBP1 and Claspin in ATR signaling), ATM and ATR activate the effector kinase CHK2 and CHK1, respectively, which then spread the signal throughout the nucleus^{96–98}. CHK1 and CHK2 decrease cyclin-dependent kinase (CDK) activity, which slows down or arrests cell-cycle progression. Meanwhile, ATM/ATR signaling promotes DNA repair through various mechanisms. Through ATM/ATR signaling, DNA repair and cell cycle progression are closely coordinated. The coordinated action of DNA repair and cell cycle controls either promotes the resumption of normal cell functioning before replication or triggers apoptosis/

cell death when normal cell functioning cannot be restored; both mechanisms act as barriers to tumorigenesis¹⁹.

Ataxia-telangiectasia (AT) is a rare autosomal recessive disorder, characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, susceptibility to bronchopulmonary disease, and lymphoid tumors. AT is caused by deficiency in the *ATM* (Ataxia-telangiectasia mutated) gene, localized on chromosome 11q22–23. ATM is a Ser/Thr protein kinase of the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family, which also includes ATR, DNA-PKcs and SMG1. ATM may have as many as 700 substrates^{99–100}, highlighting its multiple functions in various biological processes including cancer. Loss of heterozygosity in *ATM* results in reduced protein expression, however this mechanism explains only a small proportion of cancers where ATM down-regulation is observed. In sporadic cancer, which accounts for 90–95% of tumors, the probability of *ATM* gene mutations are low, whereas altered expression of ATM is frequently observed. It is therefore likely that the epigenetic modifications have an impact on ATM expression in these cases (Table 1). Initial proof for this idea came from studies using the human colon cancer cell line HCT116¹⁰¹. In this cell line, *ATM* displays aberrant promoter methylation, which inversely correlates with its low expression and low radio-sensitivity. The significance of this finding is underscored by further observations that treatment of HCT116 cells with 5-azacytidine (a DNA demethylating agent) restores expression of ATM and radio-sensitivity¹⁰¹. *ATM* is also epigenetically silenced in primary cancers. For example, 78% of surgically removed breast tumors¹⁰² and 25% of HNSCC¹⁰³ display aberrant methylation in the *ATM* promoter region accompanied by reduced ATM.

CHK2, the mammalian homolog of the yeast Rad53 and Cds1, is located at chromosome 22q12.1, spans approximately 50 kilobases, and consists of 14 exons¹⁰⁴. *CHK2*, activated by ATM, responds primarily to DSBs. Its fundamental role is to coordinate cell-cycle progression with DNA repair and cell survival or death. Germ line mutations in the *CHK2* gene predispose to Li-Fraumeni syndrome (LFS), characterized by multiple tumors at early age with a predominance of breast cancer and sarcomas¹⁰⁵. Somatic mutations in *CHK2* exist also, although they occur in only a small subset of sporadic human malignancies, including carcinomas of the breast, lung, colon, and ovary, osteosarcomas, and lymphomas¹⁰⁶. The finding of both germ line and somatic mutations suggests that *CHK2* acts as a TSG. This is further supported by the observation that down-regulation of *CHK2* is associated with promoter methylation in sporadic cancers including lung cancer, glioma and Hodgkin's lymphoma^{107–109}. For example, DNA hypermethylation of the distal *CHK2* CGIs occurs in 28.1% of NSCLCs and 40.0% of squamous cell carcinomas, which inversely correlates with *CHK2* mRNA levels. It should be noted, however, that observations in breast, colon, and ovarian cancers, do not support a causative link between DNA methylation and gene expression of *CHK2*^{110–111}.

2. DNMT1 and MMR

The function of the MMR pathway is to correct base substitution mismatches and insertion-deletion mismatches generated in newly replicated DNA¹¹². Deficiencies in or inactivation of this pathway have profound biological consequences. Loss of MMR activity is attributed to the initiation and promotion of multistage carcinogenesis¹¹³. A growing number of reports have demonstrated that loss of DNMT1 function has a significant impact on MSI - a hallmark of MMR efficiency, suggesting it has a role in the MMR pathway (Fig. 1). Using genetic screens in *Blm* deficient embryonic stem (ES) cells, *Dnmt1* was identified as an MMR modifier gene. *Dnmt1* deficiency in murine ES cells results in a 4-fold increase in the MSI rate¹³. Further support for this finding comes from several other laboratories^{14–17, 114}. DNMT1 deficiency enhances microsatellite mutations for both integrated reporter

genes^{13–14, 16–17} and endogenous repeats¹⁵. This finding holds true for both ES cells and somatic cells. In a murine ES cell line with homologous deletion of *Dnmt1*, the stability of five endogenous microsatellite repeats (two mononucleotide and three dinucleotide), exhibiting instabilities in mismatch repair-deficient cells were analyzed. A significantly higher frequency of instability was detected at three of the five markers in *Dnmt1*^{−/−} ES cells compared to the wild-type ES cells¹⁵. The slippage rate of a stable reporter gene was also monitored. Dnmt1 deficiency led to a 7-fold higher rate of microsatellite slippage in *Dnmt1*^{−/−} ES cells compared to wild type cells¹⁴. Notably, no DNA methylation in the region flanking the reporter gene was discovered, regardless of Dnmt1 status, suggesting that the effect of Dnmt1 on MMR was not at the level of DNA methylation¹⁴. Enhanced MSI is associated with higher levels of histone H3 acetylation and lower MeCP2 binding at regions near the assayed microsatellite, suggesting that Dnmt1 loss decreases MMR efficiency by modifying chromatin structure. CAG repeat expansions are closely associated with human age-related diseases including twelve neurodegenerative disorders. Repeat instability induced by CAG repeat expansion requires the mismatch repair components^{16, 115}. DNMT1 deficiency induces destabilization and intergenerational expansion of CAG triplet repeats¹⁶. Double knockdown of MLH1 and DNMT1, however, additively increases the frequency of CAG contraction¹¹⁴. Specific targeting of DNMT1 in hTERT-immortalized normal human fibroblasts by siRNA induces both resistance to MSI and the drug 6-thioguanine (which induces cytotoxic DNA damage due to its misincorporation opposite thymine¹¹⁶) at a CA17 reporter gene; two hallmarks of MMR deficiency. Mutation rates correspond well with DNMT1 levels, ranging from 4.1-fold in cells with 31% of the normal DNMT1 protein level, to 10-fold in cells with 12% of the normal DNMT1 protein level¹⁷. This suggests that DNMT1 regulates microsatellite stability in a dose-dependent manner. The exact underlying mechanism of how DNMT1 is involved in MSI appears complex and remains elusive. Microsatellite methylation probably provides a mechanism for length stabilization by subsequent transcriptional repression of genes containing or proximal to microsatellites with methylated CpG repeats. However, increased mutations usually occur at microsatellite repeats that do not contain any CpG sites in the repeat itself^{13, 15–16, 114} or nearby¹⁴, indicating that DNA methylation changes around microsatellite repeats, at least in some cases, are not the primary cause of the instability. Alternatively, DNMT1 might influence transcriptional repression and MSI through chromatin remodeling¹⁴.

The impact of DNMT1 on the MMR pathway is further highlighted by the observation that DNMT1 and the MMR proteins probably interact with each other through a third-party mediator (Fig. 1). The methyl CpG-binding protein MBD4/MED1 may provide a functional link between MMR and DNMT1 through protein-protein interaction. MBD4, which possesses glycosylase repair activity for G:T mismatches, is involved in NER as well as MMR. MBD4 binds MLH1 via its C-terminal glycosylase domain^{117–118}. Deletion of *Mbd4* in MEFs induced destabilization of MMR proteins and conferred resistance to antitumor drugs including 5-FU and platinum¹¹⁹. MBD4 and TDG have functional overlap and they interact with the *de novo* methyltransferases DNMT3A and DNMT3B^{120–121}. MBD4 also interacts with maintenance methyltransferase DNMT1 via its N-terminal MBD domain¹¹⁸. Based on a combination of immunoprecipitation and GST-pull down experiments in mouse, rat and *Xenopus*, a minimal domain of approximately 70 amino acids in the N-terminal targeting sequence region of DNMT1 was shown to be required for MBD4 to bind to DNMT1¹¹⁸, which overlaps with a region in rat DNMT1 that interacts with MECP2¹²². Through interacting directly with both DNMT1 and MLH1, MBD4 recruits MLH1 to heterochromatic sites that are coincident with DNMT1 localization¹¹⁸. Similarly, MBD4/MLH1 accumulates at DNA damage sites where DNMT1 is recruited after laser microirradiation¹¹⁸. Loss of DNMT1 induces p53-dependent apoptosis, which can be rescued by inactivation of p53¹²³. The MBD4/MLH1 complex also mediates the apoptotic response

to DNMT1 depletion¹¹⁸. Colocalization of these proteins at damaged regions implies that they function coordinately in the cellular decision to repair the lesion or activate apoptosis. Like MBD4, PCNA may act as a mediator between MMR and DNMT1 because of its direct interaction with both systems. PCNA interacts with multiple components of the MMR pathway including MSH6, MSH3, and MLH1. Disruption of this interaction confers a mismatch repair defect *in vivo* and *in vitro*^{124–126}. Both MSH6 and MSH3 co-localize with PCNA at replication foci during S-phase¹²⁷. MLH1 is recruited to damage sites where PCNA and DNMT1 also accumulate, although with slower kinetics than DNMT1^{118, 128}. The recruitment of DNMT1 to both the replication fork and DNA damage sites is through a direct interaction with PCNA and possibly CHK1 and the 9-1-1 complex as well^{21, 24}. However, there is no report showing that PCNA, MLH1 and DNMT1 colocalize together, implying that PCNA might interact with each protein at a different time. Nonetheless, the functional mechanisms of whether and how these factors are orchestrated in response to DNA damage remains to be further investigated.

Most recently, DNMT1 deficiency has been shown to induce the depletion of multiple repair factors at the protein level (Fig. 1)¹⁷, highlighting its importance not only in MMR efficiency, but also in DDR signaling. In normal human fibroblasts and CRC cell lines, DNMT1 knockdown leads to a matching decrease in MLH1 at the protein, but not the mRNA level¹⁷. Loss of MLH1, however, does not lead to expression changes in DNMT1¹⁷. Promoter hypermethylation of *MLH1*, although frequently observed in sporadic colon cancers³⁹, does not appear to be the cause leading to gene inactivation in the context of DNMT1 deficiency. *MLH1* hypermethylation in DNMT1-deficient cells was further ruled out using a bisulfite pyrosequencing assay¹⁷. Further observations suggest that DNMT1 deficiency affects the steady-state levels of a number of repair proteins, including MSH2, MSH6, and PMS2, as well as MBD4¹⁷. Loss of multiple MMR components in DNMT1 hypomorphic cells indicates that DNMT1 might play an indirect role in the stabilization or proteolytic cleavage of these proteins, rather than directly interacting with each of them. It is documented that DNMT1 deficiency activates the DDR, which leads to cell cycle arrest^{21, 123} and the triggering of cell-death pathways¹²³ that may result in cleavage of proteins including MLH1¹²⁹, which might account for MMR protein depletion after DNMT1 knockdown. Loss of DNMT1 activates ATM/ATR, which normally phosphorylate H2A.X leading to focal accumulation of γ H2A.X, a hallmark of DDR²¹. If excessive damage exists, p53-dependent¹²³ and other cell-death pathways are activated to maintain genomic integrity. Elevated γ H2A.X levels in DNMT1 hypomorphic cells can be partially reduced through inhibition of ATM/ATR signaling¹⁷. However, the PARP (PAR polymerase) inhibitor DPQ also reduces the level of γ H2A.X, to an extent exceeding that observed with the ATM/ATR inhibitor caffeine. In keeping with these observations, the viability of DNMT1-depleted cells treated with DPQ is enhanced to a greater extent than treatment of cells with agents that inhibit caspases or p53¹⁷. These findings, together with the observation that PARylation increases after DNMT1 loss, clearly demonstrate that PARP is involved in the DDR and cell death process in cells deficient in DNMT1 (Fig. 1). PARP catalyzes the polymerization of ADP-ribose (PAR) units on target proteins using NAD⁺ (nicotinamide adenine dinucleotide) molecules as a donor¹³⁰. NAD⁺ depletion, induced by severe DNA damage, gives rise to mitochondrial membrane depolarization and AIF (apoptosis initiation factor) translocation. It eventually results in an activation of caspases that lead to protein cleavage and cell death. DNA repair protein MLH1¹²⁹, along with BLM1¹³¹ and ATM¹³², are preferred targets of caspases. Treatment with the PARP inhibitor DPQ, as expected, leads to an increase in full-length MLH1 protein levels in DNMT1-depleted cells¹⁷. Taken together, DDR signaling, particularly the cell death pathway mediated by PARP, may play a substantial role in regulating cleavage of MMR repair proteins in cells deficient for DNMT1 (Fig. 1).

3. DNMT1 and the DNA Damage Response

Reduction of DNMT1 levels activates a DNA damage response usually initiated by the most lethal form of DNA damage-DSBs (Fig. 1). DNMT1 deficiency also inhibits DNA replication^{22–23, 133}. It was reported that DNMT1 knockdown triggers an intra-S-phase arrest of DNA replication, independent of DNA demethylation²². Similar to the observations for DNA damage checkpoints¹³⁴, the intra-S-phase arrest is transient, disappearing after 10 days of treatment with *DNMT1* siRNA. The S-phase cells induced by DNMT1 knockdown exist in two distinct populations: 70% incorporate BrdUr, while 30% do not, consistent with the presence of an intra-S-phase checkpoint triggering cell cycle arrest¹³⁴. Cells are arrested at different positions throughout S-phase, suggesting that this response is not specific to distinct classes of origins of DNA replication. 5-aza-CdR, a nucleoside analogue, is a well-characterized and widely-used inhibitor of DNA methylation, which inhibits DNA methylation by trapping DNMT1 at the replication fork after being incorporated into DNA. 5-aza-CdR does not inhibit the *de novo* synthesis of DNMT1 protein or its presence in the nucleus. S-phase cells treated with 5-aza-CdR, which causes genome-wide demethylation, do not exhibit two distinct population distributions as observed in cells deficient in DNMT1. These results suggest that the intra-S-phase arrest is not correlated with the degree of DNA methylation, consistent with observations that DNA replication arrest following DNMT1 inhibition is probably due to a reduction in the physical presence of DNMT1 at the replication fork, rather than DNA demethylation¹³³. As discussed above, the cell cycle distribution in DNMT1 knockdown cells resembles the transient intra-S-phase arrest in DNA replication that is evoked by genotoxic insults^{135–137}. In addition, DNMT1 inhibition also leads to the induction of a set of genes that are implicated in the genotoxic stress response including *p21*¹³³, *p53*¹²³, and the growth arrest DNA damage inducible 45β gene (*GADD45β*)²². These results imply that DNMT1 is linked to DNA damage repair machineries to maintain chromosome integrity via blocking DNA replication, a notion further strengthened by observations that DNMT1 knockdown activates the checkpoint pathways in a ATR-dependent manner²³. Upon DNMT1 depletion, CHK1 and CHK2, key proteins in ATM/ATR signaling, are phosphorylated, which in turn induce phosphorylation and degradation of cell division control protein 25 A (CDC25A) as well as CDC25B²³. As a consequence, the capacity for loading CDC45, an essential factor for DNA replication¹³⁸, onto replication forks is decreased, resulting in replication arrest. DNMT1 knockdown also induces the formation of histone γH2A.X foci, a hallmark of the DNA DSB response. The response elicited by DNMT1 knockdown is blocked by siRNA mediated-depletion of ATR, suggestive of its ATR-dependency. Further support for the importance of ATR came from the finding that the cellular response to DNMT1 depletion is markedly attenuated in cells derived from a patient with Seckel syndrome, a disorder due to ATR deficiency²³. However, it is not clear whether ATM, another key transducer like ATR in the checkpoint pathway, is involved in the process or not. DNA demethylating agents do not trigger the stress response like genetic DNMT1 depletion does²³. Moreover, this response is abolished by ectopic expression of either wild-type DNMT1 or a mutant form of DNMT1 lacking the catalytic domain²³, suggesting that loss of catalytic activity of DNMT1 is not driving this response. Also of importance, DNMT1 knockdown leads to very limited genomic demethylation^{22–23}, consistent with observations made in cells containing hypomorphic mutations in *DNMT1*^{139–140}. One explanation for this limited demethylation is that *de novo* DNMTs compensate for the reduction of DNMT1 activity¹³⁹. Another possibility is that DNMT1 loss triggers a checkpoint pathway (Fig. 1) to block DNA replication, preventing loss of DNA methylation in an attempt to maintain genome stability. Double knockdown of DNMT1 and ATR does indeed induce global DNA demethylation, whereas single knockdowns of either DNMT1 or ATR do not, implying that the arrest of DNA replication activated by ATR signaling following DNMT1 depletion prevents loss of DNA methylation and that blocking this response results in global loss of DNA methylation²³. Taken together,

it appears that reduction of DNMT1 levels activates ATR signaling to block DNA replication in a DNA methylation-independent manner (Fig. 1). How this response to DNMT1 reduction is initiated, however, is still uncertain. It is possible that removal of DNMT1 from replication forks disrupts fork progression and eventually results in DSBs that elicit checkpoint signaling (Fig. 1). Alternatively, the presence of low levels of hemimethylated DNA due to the absence of DNMT1 may trigger this response (Fig. 1).

Complete inactivation of DNMT1 via genetic mechanisms also activates the DNA damage response and causes genomic demethylation. The degree of demethylation, however, varies greatly depending on cellular context, ranging from 20% loss in human cancer cells¹⁴¹ to 90% loss of genomic methylation in murine ES cells⁷⁻⁸. As the principal enzyme responsible for maintaining DNA methylation, DNMT1 is essential for embryonic development and cell survival. Disruption of *Dnmt1* in mice results in loss of 90% of genomic methylation and embryonic lethality⁷⁻⁸. Murine ES cells deficient for *Dnmt1* die when introduced to differentiate⁷, mouse fibroblasts die within 2–4 cell divisions after conditional deletion in *Dnmt1*¹²³, and the human colon cancer cell line HCT116 undergoes marked apoptosis and cell death within one cell division if *DNMT1* is completely inactivated by cre-mediated conditional knockout¹⁴¹⁻¹⁴². Notably, complete inactivation of DNMT1 triggers the DNA damage response before cells die¹⁴¹. Deletion of *DNMT1* activates p53^{123, 141}, a target of ATM whose phosphorylation correlates with accumulation of p53 in response to DNA damage¹⁴³. Disruption of both alleles of *DNMT1* leads to activation of the G2/M checkpoint and G2 arrest, as verified by the presence of phosphorylated ATM and γ H2A.X at discrete nuclear DNA damage foci¹⁴¹. Further support for checkpoint activation comes from the finding that treatment of cells with an ATM/ATR inhibitor, caffeine, facilitates mitotic entry and cell death in *DNMT1* null cells¹⁴¹. Most of these cells, however, eventually escape G2 arrest and re-enter interphase with their unrepaired DNA, resulting in severe chromosomal and mitotic abnormalities (mitotic catastrophe)¹⁴¹. Thus far, the mechanisms by which DNMT1 inactivation leads to activation of the DNA damage repair remains elusive. In the complete absence of DNMT1, DNA may become more fragile owing to reduced methylation and/or defective chromatin structure in critical regions of the genome, leading to activation of DNA damage signaling (Fig. 1)¹⁴². Alternatively, the accumulation of hemimethylated DNA in *DNMT1* mutant cells may be recognized as damage and trigger the damage response (Fig. 1). Both of these possibilities are consistent with the observation that agents that affect overall chromatin structure without damaging DNA also activate ATM¹⁴⁴. Nonetheless, it cannot be excluded that oncogene activation or gene mutations initiate the DNA damage response, as *Dnmt1*-deficient ES cells exhibit significantly increased mutation rates, particularly in the form of deletions and mutations¹⁴⁵.

Recruitment of DNMT1 to sites of DNA damage has been observed by our laboratory^{21, 146} and others²⁴, providing compelling evidence to support the notion that DNMT1 is directly involved in DNA damage repair (Fig. 1). Immediately after laser microirradiation-induced DSBs, an accumulation of DNMT1 and PCNA occurs at the damage sites in S and non-S phase cells, colocalizing with γ H2A.X - a marker of DSBs. Recruitment of DNMT1 to damage sites is dependent on its interaction with PCNA through its PCNA binding domain (PBD)^{21, 24}, but is independent of its catalytic activity²¹. In addition to PCNA, DNMT1 also interacts with other components of the DNA damage machinery including CHK1^{21, 146} and the 9-1-1 complex²¹. PCNA, along with CHK1 and 9-1-1, is essential for DNMT1's recruitment to DNA damage sites. After recruitment to damaged regions, DNMT1 modulates the rate of ATR signaling and is essential for suppressing abnormal activation of the DNA damage response in the absence of exogenous damage²¹. Taken together, these data have revealed a direct link between DNMT1 and the DNA damage repair process.

PCNA mediates recruitment of DNMT1, not only to DNA replication sites, but also to DNA damage sites. The DNMT1-PCNA interaction implies that the role of DNMT might be to restore epigenetic information after damage repair. However, recent studies demonstrate that this interaction is not essential for maintaining DNA methylation^{5, 147}. Furthermore, the observation²¹ that DNMT1 is very rapidly recruited and retained only transiently, likely before re-synthesis is completed, suggest that genomic methylation is not the main function of DNMT1 at these sites, at least in the early part of the DNA damage response. The recruitment kinetics of WT *DNMT1* and *DNMT1* with a point mutation in the catalytic domain are almost identical²¹. CHK1/CHK2 activation and γ H2A.X foci formation induced by DNMT1 deficiency are rescued by expression of a catalytically inactive form of DNMT1²³. Therefore, although the possibility that DNMT1 participates in the restoration of DNA methylation patterns during damage repair cannot be excluded, it seems more likely that DNMT1 functions in sensing and/or mobilizing the response to certain forms of DNA damage (Fig. 1).

In summary, both DNMTs and DNA damage repair systems have evolved to maintain genomic integrity and disruption of these pathways contributes to the development of cancer¹⁹. Therefore, we have examined and outlined the interaction of DNMTs and DNA methylation with DNA damage repair systems, and have discussed possible mechanisms for how the two systems may function coordinately to deal with DNA damage. Promoter methylation, catalyzed by DNMTs, plays an established role in silencing key genes in multiple DNA damage repair pathways; inactivation of these pathways may predispose to a large array of tumors²⁰. These findings are consistent with observations that TSGs are frequently silenced via epigenetic mechanisms in cancer cells. Unexpectedly perhaps, more recent observations strongly suggest that DNMTs, particular DNMT1, are directly involved in DNA damage repair systems via what is likely to be a DNA-methylation-independent mechanism^{17, 21–23, 141}. The exact nature of the links between the DNMTs, DNA methylation and DNA damage repair systems are likely complex and remain to be further investigated. A more thorough understanding of these links will not only help dissect the mechanisms of tumor development, but also identify new anti-tumor targets and therapeutic strategies.

Acknowledgments

Work in the Robertson laboratory is supported by NIH grants R01CA116028, R01CA114229, and the Georgia Cancer Coalition (KDR). KDR is a Georgia Cancer Coalition Distinguished Cancer Scholar.

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Fig. 1. Impact of DNMT1 on MMR and DDR

DNMT1 may promote stabilization of microsatellites via methylation of CpG repeats and it also interacts with DNA repair proteins via third-party mediators (e.g. MBD4 and PCNA). Moreover, deficiency in DNMT1 leads to activation of PARP signaling, eventually resulting in MMR protein cleavage. DNMT1 is also closely associated with DDR. Inactivation of DNMT1 may induce several changes to DNA and/or chromatin including increased DNA fragility, disruption of replication foci, and accumulation of hemimethylated DNA, which may be recognized as “damage” and activate the DDR. Strong support for a direct link between DNMT1 and DDR comes from the identification of several protein-protein interactions involving DNMT1 and DDR proteins. DNMT1 is recruited to sites of DNA damage via its interaction with PCNA and 9-1-1. DNMT1 is also capable of binding CHK1 and p53, which promote cell cycle arrest and apoptosis, respectively.

Table 1

Genes in DNA Damage Repair Pathways that are Hypermethylated

Repair Pathway	Methylated Gene	Cancer Type	Samples Studied	Samples Methylated	Methylation Frequency (%)	References
MMR	MLH1	sporadic CRC (MSI+)	110	67	61	41–44 ^a
		sporadic CRC (MSI–)	128	38	30	42–43 ^a
		sporadic early-onset CRC	110	55	50	45
		NSCLC	77	43	56	32
		acute myeloid leukemia	177	11	6	34–36 ^a
		ovarian cancer	672	72	11	33
		oral squamous cell carcinoma	99	8	8	29
		HNPCC	179	2	1	39–40 ^a
		gastric cancer	306	58	19	30–31 ^a
		HNSCC	49	14	29	37
	MSH2	NSCLC	14	4	29	32
		gastric cancer	200	27	14	30
		ovarian cancer	56	29	52	46
		sporadic CRC	36	1	3	47
		HNPCC	46	11	24	48
		gastric cancer	200	25	13	30
		breast cancer	33		92–95 ^a /20 ^o	50
		multiple myeloma	KAS-6/1 cell line			52
		CRC	339		24 ^a /14 ^o	53
		thyroid cancer	38	2	5	54
NER		bladder cancer	37	12	32	56
		glioma	32	unknown		57
		gastric cancer	25	unknown		60
		multiple myeloma	KAS-6/1 cell line			61
		NSCLC	98	29	30	69
HR	BRCA1	sporadic ovarian cancer	81	12	15	66
		sporadic breast cancer	190	24	13	64,70 ^a

Repair Pathway	Methylated Gene	Cancer Type	Samples Studied	Samples Methylated	Methylation Frequency (%)	References
NHEJ ATM/ATR	BRCA2	hereditary breast cancer	162	18	11	70
		early onset gastric cancer	104	0.6	1	67
		bladder cancer	96	0.71	1	68
		breast cancer	33		59–64 ^a /10 ⁶	50
	FANCC	NSCLC	98	41	42	69
		acute lymphoblastic leukaemia	97	3	3	74
	FANCF	acute myeloid leukaemia	143	1	1	74
		HNSCC	89	13	15	75
	FANCL	NSCLC	158	22	14	75
		cervical cancer	91	27	30	76
ovarian cancer		53	7	13	77	
acute lymphoblastic leukaemia		97	1	1	74	
Others	XRCC5	NSCLC	98	19	19	69
		HNSCC	100	25	25	101
	ATM	breast cancer	23	18	78	100
		CRC	HCT116 cell line			99
	CHK2	NSCLC	139	39	28	106
		glioma	5	5	100	107
	MGMT	oral squamous cell carcinoma	99	40	40	29
		gastric cancer	200	50	25	30
		CRC	36	14	39	80
		HNSCC	21	6	29	80
NSCLC		34	10	29	80	
lymphomas		61	15	25	80	
glioma		140	54	39	80	
gastric cancer		38	10	26	91	
WRN	CRC	182	69	38	91	
	NSCLC	56	21	38	91	
	prostate cancer	20	4	20	91	
	breast cancer	58	10	17	91	
	thyroid cancer	32	4	13	91	

Repair Pathway	Methylated Gene	Cancer Type	Samples Studied	Samples Methylated	Methylation Frequency (%)	References
		non-Hodgkin lymphoma	118	28	24	91
		acute lymphoblastic leukemia	21	2	10	91
		acute myeloblastic leukemia	36	3	8	91
		chondrosarcomas	15	5	33	91
		osteosarcomas	27	3	11	91

* Mean methylation level (%) in cancer vs © mean methylation level (%) in control

α Indicates references from which methylation data derived from similar samples was pooled for this summary

CRC-colorectal cancer; NSCLC-non-small cell lung cancer; HNPCC-hereditary non-polyposis colorectal cancer; HNSCC-head and neck squamous cell carcinoma