Modern Pathology (2007) 20, 711–721

www.modernpathology.org

DNA methylation in breast and colorectal cancers

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DNA methylation is one of several epigenetic changes observed in cells. Aberrant methylation of tumor suppressor genes, proto-oncogenes, and vital cell cycle genes has led many scientists to investigate the underlying cellular mechanisms of DNA methylation under normal and pathological conditions. Although DNA methylation is necessary for normal mammalian embryogenesis, both hypo- and hypermethylation of DNA are frequently observed in carcinogenesis and other pathological disorders. DNA hypermethylation silences the transcription of many tumor suppressor genes, resulting in immortalization of tumor cells. The reverse process, demethylation and restoration of normal functional expression of genes, is augmented by DNA methylation inhibitors. Recent studies suggest that DNA hypomethylation may also control gene expression and chromosomal stability. However, the roles of and relationship between hypomethylation and hypermethylation are not well understood. This review provides a brief overview of the mechanism of DNA methylation, its relationship to extrinsic stimulation including dietary intake and aging, and of abnormally methylated DNA in breast and colorectal cancers, which could be used as prognostic and diagnostic markers.
Modern Pathology (2007) **20,** 711–721; doi:10.1038/modpathol.3800822; published online 27 April 2007

Keywords: breast cancer; chromosomal stability; colorectal cancer; DNA methylation; hypomethylation; hypermethylation

During translation and transcription, DNA exerts its effects in cells through regulatory mechanisms including mRNA stabilization, transcription, and epigenetic changes.[1] The pattern of altered gene expression or epigenetic change is of major importance in common malignancies.[2,3] Methylation of the DNA, histone deacetylation, ubiquitination, and phosphorylation are examples of epigenetic change.[4] DNA methylation, unlike the other epigenetic changes, does not alter the nucleotide sequence.

Most cytosine-phosphoguanine (CpG) dinucleotides are unevenly distributed throughout the genome and remain in short stretches or clusters (500–2000 bp), called CpG islands.[5–7] These islands are located in the promoter region and are found in half of all human genes.[8] In mammals, DNA methylation occurs after replication, when a methyl group (CH_3) is added to the 5′ position of cytidyl residues in the dinucleotide sequence CpG[9,10] (Figure 1). Endonucleases, which normally degrade foreign DNA, regulate gene expression by silencing genes when the CpG is methylated.[11] CpG islands remain unmethylated in housekeeping genes and methylated or silenced in other genes.[12]

DNA is methylated by DNA methyltransferases (DNMTs), which transfer the methyl group from *S*-adenosylmethionine (SAM) to generate patterns of genomic methylation that silence genes[13–15] (Figure 1). The DNMTs known to date are DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L.[15,16] All methyltransferases have homology and different functions. DNMT1 maintains established methylation patterns in hemi-methylated genes by copying methylation patterns from the parent strand to the daughter and is expressed during the S-phase.[17] DNMT2, a small protein of 391 amino acids, is known to have weak DNA methyltransferase activity.[18] DNMT3a and DNTM3b, referred to as *de novo* methyltransferases, methylate unmethylated DNA. They initiate normal DNA methylation during embryonic development.[19] DNMT3L does not bind to SAM, but increases the binding of DNMT3a to SAM. Since DNMT3L-deficient mice are sterile, DNMT3L is likely to be essential in the methylation process.[19]

Overexpression of DNMTs can be lethal in animals as well as in human cancers.[20] DNMTs are involved in the downregulation of tumor suppressor

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Received 21 December 2006; revised 29 March 2007; accepted 03 April 2007; published online 27 April 2007

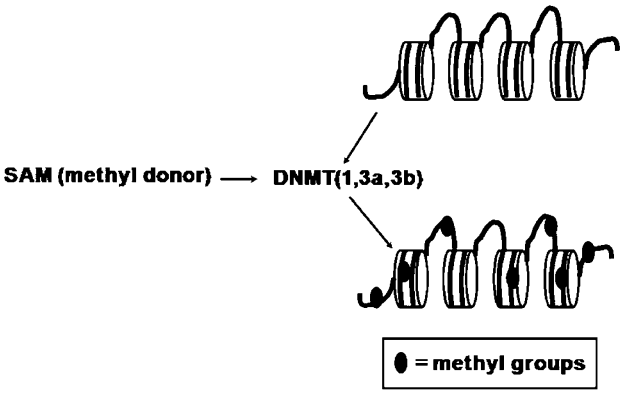

Figure 1 DNA methylation. SAM and other methyl donors bind to DNMTs to methylate the DNA that is tightly wrapped around the histones.

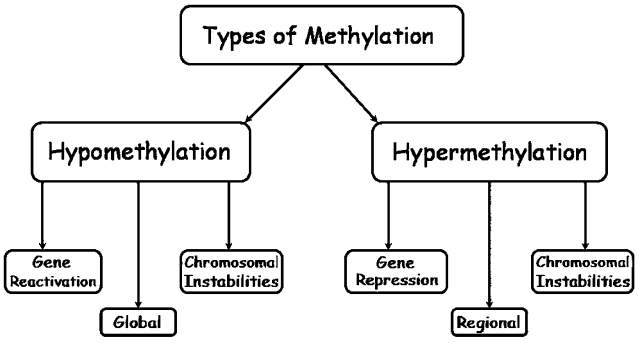

Figure 2 Two types of aberrant methylation, which elucidate certain actions.

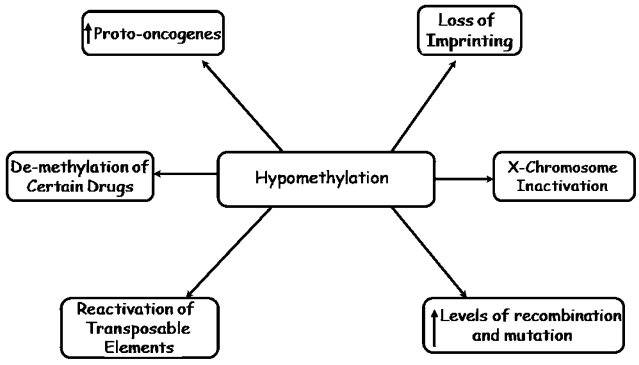

Figure 3 Known functional outcomes of DNA hypomethylation.

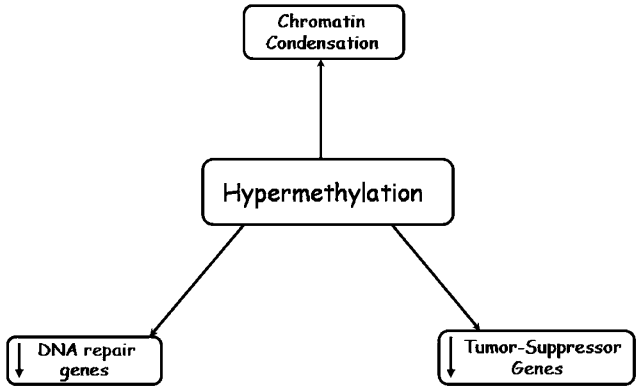

Figure 4 Known functional outcomes of DNA hypermethylation.

genes and stimulation of proto-oncogenes.[21,22] Although DNMTs are important in DNA methylation, several findings indicate that DNMTs are not essential for the promotion of carcinogenesis.[23–25]

Hypo- and hypermethylation

When gene expression is altered due to DNA methylation, it is usually categorized as due to hypo-methylation or hypermethylation (Figure 2). DNA hypomethylation is associated with gene reactivation and chromosomal instabilities.[26,27] Functional outcomes of hypomethylation include the upregulation or overexpression of transcription of proto-oncogenes, increased recombination and mutation, X-chromosome inactivation, loss of imprinting, reactivation of transposable elements, and demethylation of xenobiotics[10,28,29] (Figure 3). Activation of proto-oncogenes, reactivation of transposable elements, and loss of imprinting of genes are the results of hypomethylation and all promote cancer.[30]

When CpG islands are hypermethylated, the activity of the regulatory proteins that promote transcription is restricted due to the tightly packed nucleosomes.[8] DNA hypermethylation is involved in gene repression and chromosomal instabilities.[31] Results of hypermethylation include suppression of tumor suppressor genes, chromatin condensation, and suppression of DNA repair genes (Figure 4). Tumor suppressor genes contain unmethylated CpG islands in their promoter regions and are methylated in various malignancies.[32–34] Both types of methylation occur simultaneously in various sporadic cancers and affect the function of human tumor suppressor genes and proto-oncogenes.[35]

Detection of DNA methylation

Currently, several methods are used to detect methylated DNA (Table 1).

Methylated DNA can be detected by converting unmethylated cytosine residues to uracil using sodium bisulfite modification, followed by polymerase chain reaction (PCR) to identify the unmethylated nucleotides.[36] Methylation-specific PCR (MSP) detects the methylation of CpG islands, but with higher specificity and sensitivity.[37] Quantitative real-time PCR (MethyLight or QM-MSP) is used to detect low levels of methylation that cannot be distinguished using MSP.[38,39]

Table 1 Methods of detection for methylated DNA sequences

Method	*Acronym*	*Function*	*Reference*
Methylation-specific PCR	MSP	Non-quantitative; quick way to determine if gene is methylated after sodium bisulfite modification	36,37
Quantitative multiplex methylation-specific PCR	QM-MSP or MethyLight	Quantitatively detects methylated alleles and can differentiate between monoallelic and biallelic sequences	38,39
McrBC-methylation-sensitive-arbitrarily-primed PCR	McrBC-msAP-PCR	Detects band intensity of methylated sites using enzyme McrBC	40
Methylated CpG island recovery assay	MIRA	Array based; uses genomic DNA to detect methylated sites	41,42
Combined bisulfite restriction analysis	COBRA	Combines PCR and restriction enyzme analysis to detect methylated sites	43
Differential methylation hybridization	DMH	Array based; can analyze > 50 000 genomic fragments for methylation at once	44
Microarray methylation assessment of a single mass	MMASS	Array based; compares genome-wide methylated to unmethylated sequences in a single sample	45
Methylation target array	MTA	Array based; detects hypermethylation of multiple loci in a variety of tumors	46

A modification of MSP, termed McrBC-methylation-sensitive-arbitrarily-primed-polymerase-chain-reaction (McrBC-msAP-PCR) requires methylation-specific GTP-dependent restriction endonuclease, McrBC, to detect differentially methylated sites within DNA, where hypermethylation and hypomethylation is observed by DNA fingerprint band intensity.[40] The procedure for the methylated CpG island recovery assay (MIRA) requires isolated and sonicated genomic DNA, instead of sodium bisulfite.[41] PCR reactions detect CpG island methylation after the DNA is incubated with a matrix containing methyl-CpG binding domain protein-2b (MBD2b) and methyl-CpG binding domain protein 3-like-1, which bind specifically to methylated DNA sequences.[41,42] MIRA can be used to study the methylation status of a wide array of genes in cancers, such as the lung.[42] Currently, other array-based methods are being developed to screen the methylation pattern of several genes.[39]

Differential methylation hybridization (DMH) is an oligonucleotide array-based method, which determines the extent of methylation of CpG islands by comparison with a reference sample.[44] Methylation-specific restriction enzymes (*Mse*I) are used to obtain intact CpG islands. The CpG islands are fluorescently labeled and after subsequent PCR, they are hybridized to arrayed oligonucleotides that can discriminate between methylated and unmethylated alleles in regions of interest. Microarray methylation assessment of single samples (MMASS) has been shown to be more sensitive and is an optimized method for detecting the methylated and unmethylated sequences within the entire genome.[45] MMASS uses the methylation-specific enzyme, McrBC, instead of *Mse*I. It is able to detect unmethylated sequences more effectively than DMH because McrBC only cleaves methylated sequences and does not require a reference sample.[45] Methylation target array (MTA) simultaneously determines whether or not genes and CpG islands in multiple tumors are hypermethylated and can be correlated to clinicopathological features of the patient.[46] An advantage of MTA is that a single nylon filter can be used repeatedly to probe for various genes which are indicative of DNA methylation.[47]

DNA methylation and cancer: overview

Aberrant DNA methylation is one of the many potential causes for the abnormal growth of cancer cells, but it is also known to protect against intestinal cancer.[4] Different types of cancers are associated with methylation of tumor suppressor genes and proto-oncogenes, causing alterations in functional gene expression. Cancer-specific DNA methylation patterns have been detected in free-floating DNA released from dead cancer cells.[5,48] A decrease in the expression of tumor suppressor genes correlates with an increase in methylation of DNA in the promoter region.[49,50] Aberrant methylation of tumor suppressor genes in many cancers, resulting in the downregulation of transcriptional activation, has been reported.

In some cancers, both hypermethylation and hypomethylation are observed. Hypomethylation increases progressively with increasing malignancy grade in breast, ovarian, cervical, and brain cancers.[51] Breast and colorectal cancers are malignancies commonly caused by regional hypermutability or global hypomethylation.

DNA methylation and breast cancer

Many factors contribute to the pathogenesis of breast cancer, which is one of the most common malignancies among females. These factors include family history, nutrition, age, and epigenetic changes including DNA methylation. Methylation appears to be an early event in the etiology of breast

carcinogenesis, resulting in the activation of many oncogenes and silencing of tumor suppressors to promote proliferation of abnormal cells.[52,53] It is debatable, however, whether global hypomethylation or regional hypermethylation occurs first during the development of breast cancer, since the phenomena are independent processes. It is not known whether or not it is possible to inhibit carcinogenesis by inhibiting one of these processes and not the others. Numerous studies have revealed various genes, which are either hypo- or hypermethylated in breast cancer (Table 2).

Metastatic breast cancer requires the expression of multiple genes. Regional hypermethylation and global hypomethylation are involved in different stages of breast cancer.[76] Global hypomethylation could be a mechanism for late stages of breast cancer while local hypermethylation is plausible for early stages of breast cancer.[22,30] DNA methylation results in altered gene products including cell cycle regulators, steroid receptors, and cell adhesion molecules, which give rise to increased susceptibility to tumor development and decreased detoxification of carcinogens.[58] Alterations in the breast cancer susceptibility gene product (BRCA) accounts for half of the inherited breast carcinomas.[77] Its methylation is observed in breast and ovarian cancers, but not in colon and liver cancers, or

Table 2 Genes methylated in breast cancer

Gene	*Description*	*Hypo/hyper*	*Reference*
1-SYNU-CLEIN	Human breast cancer-specific gene 1	Hypo	54
c-myc	C-myelocytic leukemia	Hypo	55
MAGE	Melanoma-associated antigen	Hypo	56
NOEY2/ARHI	Ras homolog member 1	Hypo	52
Sat2	Satellite 2	Hypo	57
SATR1/SATR2	Satellite repeat 1/satellite repeat 2	Hypo	57
UPA	Urokinase plasminogen activator	Hypo	54
14-3-3sigma	14-3-3 sigma	Hyper	58
AK5	Adenylate kinase 5	Hyper	59
AMN	Amnionless homolog	Hyper	59
APC	Adenomatous polyposis coli	Hyper	54
BRCA1	Breast cancer	Hyper	60
CDH1	Cadherin 1	Hyper	61
CDKN2A	Cyclin-dependent kinase inhibitor 2A	Hyper	17
DAPK1	Death-associated protein kinase 1	Hyper	54
DCC	Deleted in colorectal carcinoma	Hyper	59
DSC3	Desmocollin 3	Hyper	62
ER	Estrogen receptor	Hyper	8
FOXA2	Forkhead box A2	Hyper	59
GJB2	Gap junction protein, beta 2 (Connexin 26)	Hyper	59
GSTP1	Glutathione *S*-transferase pi	Hyper	32
HIC-1	Hypermethylated in cancer 1	Hyper	54
HIN-1	Hairpin induced 1	Hyper	63
HME-1	Human epithelial cell marker 1	Hyper	64
HOXD11	Home box D11	Hyper	59
KLK6	Kallikrein 6	Hyper	65
KLK10/NES1	Kallikrein 10	Hyper	66
LATS1/LATS2	Large tumor suppressor 1 and 2	Hyper	67
LKB1/STK11	Serine/threonin protein kinase 11	Hyper	68
MGMT	Methylguanine methyltransferase	Hyper	69
NORE1	Novel Ras effector 1	Hyper	70
p14ARF	p14 alternate reading frame	Hyper	71
P16INK4a	p16 INK 4a	Hyper	71
p57KIP2	Cyclin-dependent kinase inhibitor 1C	Hyper	72
PCDH10	Protocadherin 10	Hyper	59
PR	Progesterone receptor	Hyper	54
Rad9	Rad9 homolog	Hyper	73
RASSF1A	Ras-association domain family protein 1A	Hyper	54
RUNX3	Human Runt-related transcription factor gene 3	Hyper	74
SIM1	Single-Minded homolog 1	Hyper	59
TDH	L-threonine dehydrogenase	Hyper	59
TIMP-3	Tissue inhibitor of metalloproteinases-3	Hyper	54
TMS1	Target of methylation-induced silencing 1	Hyper	75
Tropomyosin	Tropomyosin	Hyper	59
TSPAN-2	Tetraspan 2	Hyper	59
Twist	Twist	Hyper	63
WT-1	Wilms tumor 1	Hyper	54
XT3	X transporter protein 3	Hyper	59

leukemia indicating a tissue-specific process.[58] The frequency of methylation of this gene product is 38.5% in sporadic breast cancer.[60] Patients with a HER2/Neu-positive tumor indicate a highly aggressive breast cancer that requires special treatment, since it is amplified in 30% of invasive breast carcinomas.[55] DNA methylation is prevalent in the highly aggressive HER2/Neu-positive breast cancers; this gene is amplified in 30% of the cancers.[55] Increased aberrant methylation of steroid receptor genes and glycoproteins, such as progesterone receptor (PR) and E-cadherin, respectively, are associated with Her2/Neu-positive cancers. Hypermethylation of the GC-rich region and loss of expression in about 80% of invasive lobular carcinomas and lobular carcinoma *in situ* indicate the importance of the methylation of the CDH1 promoter in the pathogenesis of breast cancer.[78] Although many mechanisms, including mutation and loss of heterozygosity (LOH), are attributed to the down-regulation of CDH1 in breast cancer, CDH1 promoter methylation is the mostly likely cause.[78,79]

The current criteria for detection and prognosis of breast cancer include an abnormal breast biopsy, tumor size, histological grade, estrogen and progesterone receptor status, and presence of the HER2/Neu oncogene.[80,81] Breast cancer can also be diagnosed by detecting the various aberrantly methylated genes. MSP is currently being used to detect the methylation status of various genes in breast biopsy tissues samples.[59] Presence of methylated DNA in the nipple duct lavage fluids, needle aspirates of the breast, and molecular staging of sentinel lymph nodes are also used to predict breast cancer development.[58] Ductal carcinoma *in situ* (DCIS), the most frequent breast cancer, can be detected early by observing the methylation of a panel of tumor suppressor or other cancer genes.[82,83] A 60-sample study with ductal lavage fluid from patients with a high risk of developing breast cancer and patients with breast cancer revealed that a nine-gene panel detection system using quantitative methylation-specific polymerase chain reaction (QM-MSP) can detect the rate of cancer cells more effectively than cytological and histological studies alone. Thus, earlier detection of breast cancer formation is possible.[84] Aberrant methylation of four genes was detected by QM-MSP in the plasma DNA of patients with breast cancer and tumors were successfully detected in eight of 24 patients with early-stage breast cancer.[85] Loss of MGMT (*O*(6)-methylguanine-DNA methyltransferase) was found to be associated with DNA methylation in a subset of breast cancers.[86] Although DNMT3b, a *de novo* methyl transferase, is overexpressed in 30% of breast cancers, its expression alone was not considered to be a prognostic factor for breast cancer progression.[87] Such studies highlight the importance of identifying specific methylated genes for diagnostic purposes, and for monitoring the efficacy of therapeutic modalities.

DNA methylation and colorectal cancer

Colorectal carcinoma is the third most common cancer in developed countries.[88] Although age and other demographic and environmental features, including gender, weight, nutritional intake, and alcohol consumption, are prognostic of colorectal cancer, epigenetic alterations are also causal.[89] Aberrant methylation is gradually acquired in the early stages of colorectal carcinoma.[90] As in breast cancer, both hypomethylation and hypermethylation of genes occur in colorectal cancer[91,92] (Table 3**)**. The genes for p53 and for retinoic acid receptor (RAR) are hypermethylated in colorectal cancer.[108] A study with 65 colorectal carcinoma tissues demonstrated hypermethylation of the gene for the cell cycle regulatory protein, cyclin A1, in all cases, and for cadherin-13 in 65% cases.[96] Methylation did not correlate significantly with any clinicopathological feature, and changes in methylation appeared in an early phase of colon carcinogenesis.[96]

Aberrant methylation of some genes, including those for estrogen receptor α (ERα) and myoblast determination 1-protein (MYOD), correlate with aging and the prognosis of colorectal cancer.[109] UDP-glucoronosyltransferase (UGT1A1) gene expression is silenced and transcriptional activity is completely repressed in colon cancer cells due to direct methylation of its promoter region.[97] Treatment with either the inhibitors of histone deacetylase or demethylating agents restores normal expression of UGT1A1 in hypermethylated cells but has no effect on hypomethylated cells.[97]

Rhee *et al*[100] reported that DNA methyltransferases, DNMT1 and DNMT3b, are hypermethylated in colorectal cancer. Other studies suggest that methylation depends upon the type of cancer and that colorectal cancer can progress in the absence of DNMT1, as seen in the SW48 colorectal cancer cell line.[108,110] However, methylation can be reinitiated by introducing DNMT1 to the SW48 colon cancer cells lacking DNMT1 and DNMT3b.[111] Therefore, DNMT1 is potentially important in the hypermethylation of CpG islands in the promoter region of many genes in human cancer cells.

Colorectal tumors are often identified by the level of their microsatellite instability (MSI), which is a defect in the ability of repairing mistakes during DNA replication. MSI, stratified as MSI high, MSI low, and MSI stable, is commonly correlated with the degree of the colorectal cancer methylator phenotype (CIMP) when diagnosing colorectal cancer at the molecular level.[112] CIMP is distinguished as CIMP(+) or CIMP(−), although the existence of CIMP is still controversial. Supporters suggest an association with microsatellite instability (MSI) and proximal location of the colonic tumor.[113] A study with 106 primary colorectal tumors, however, did not support the existence of CIMP in human colorectal cancer and, consequently, it was regarded as a statistical artifact.[114] Colorectal cancer cannot be

Table 3 Genes methylated in colorectal cancer

Gene	*Description*	*Hypo/hyper*	*Reference*
CDH13	E-cadherin 13	Hypo	6
ER	Estrogen receptor	Hypo	8
hMLH1	MutL homolog 1	Hypo	93
MBD2	Methyl CpG binding protein	Hypo	94
MINT1	Methylated in tumor 1	Hypo	95
MINT3	Methylated in tumor 3	Hypo	95
SFRP1	Secreted frizzled-related protein 1	Hypo	96
UGT1A1	UDP-glucoronosyltransferase	Hypo	97
APC	Adenomatous polyposis coli	Hyper	54
CDKN2a	also known as p16	Hyper	93
CTDSPL	Carboxy-terminal domain small phosphatase like	Hyper	98
CIP1	Cyclin-dependent kinase inhibitor 1B	Hyper	99
COX2	Cyclooxygenase 2	Hyper	96
Cyclin A1	Cyclin A1	Hyper	96
DNMT1	DNA methyltransferase1	Hyper	100
DNMT3b	DNA methyltransferase 3b	Hyper	100
GATA-4/GATA-5	Transcription factor GATA-4	Hyper	101
HIC1	Hypermethylated in cancer 1	Hyper	54
HME-1	Human epithelial cell marker 1	Hyper	102
KCNK15	Potassium channel subfamily K member 15	Hyper	98
MAGEA1	Melanoma antigen, family A, 1	Hyper	96
MGMT	*O*(6)-methylguanine-DNA methyltransferase	Hyper	93
MS	Methionine synthase	Hyper	103
MTHFD1	Methylenetetrahydrofolate dehydrogenase	Hyper	103
MYOD-1	Myoblast determination 1-protein	Hyper	103
N33	Tumor suppressor candidate 3	Hyper	96
p14ARF	p14 alternate reading frame	Hyper	71
p16INK4a	p16 INK 4a	Hyper	104
p53	p53	Hyper	38
PTEN	Phosphatase and tensin homolog	Hyper	96
RAR	Retinoic acid receptor	Hyper	96
RASSF1A	Ras-association domain family protein 1A	Hyper	69
SFRP2-5	Secreted frizzled-related protein 2,3,4,5	Hyper	105
SLIT1/SLIT3	Slit homolog 1 and 3	Hyper	106
TFF1-3	Trefoil factor 1, 2, 3	Hyper	101
TIMP3	Tissue inhibitor of metalloproteinase 3	Hyper	96
TROPOMYOSIN	Tropomyosin	Hyper	96
TS	Thymidylate synthase	Hyper	103
UCHL1	Ubiquitin carboxy-terminal hydrolase 1	Hyper	107
UGT1A1	UDP-glucoronosyltransferase	Hyper	97
VHL	von Hippel–Lindau syndrome	Hyper	8
WNT9A	Wing-type member 9A	Hyper	98

identified on the basis of tumor methylation status and CIMP alone.

Hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) accounts for 2–4% of all colorectal cancers and aberrant methylation of the mismatch repair genes, human mutL homolog 1 (hMLH1) or hMLH2, are the basis for the cancer.[115] The combination of MSI-H and CIMP(−) is commonly observed in HNPCC.[112] In a study with 97 colorectal adenoma cases, hMLH1 methylation was more frequently observed in overweight or obese patients.[93] High-level MSI sporadic colon cancer and HNPCC share histological features, proximal tumor location, and presence of tumor-infiltrating lymphocytes. They differ, however, in having widespread promoter hypermethylation of specific genes such as hMLH1 and BRAF.[112] Fewer methylated genes are found in HNPCC than in high-level MSI colorectal tumors.[116]

Inhibitors of DNA methylation and demethylators

DNA methylation is a reversible process in which genes can be demethylated and restored to their original expression and function. DNA methylation inhibitors have been investigated as anticancer agents, since they block the activity of DNMTs and thus activate tumor suppressor genes[54] **(**Figure 5**)**. The antisense oligonucleotide to human DNMT1, MG98, acts as a DNA methylation inhibitor and downregulates the activity of DNMT1. It has had promising results in clinical trials in treating cancers of the head and neck.[117] Recently, two novel inhibitors, NSC303530 and NSC401077, were shown to inhibit DNMTs *in vitro* and *in vivo* by blocking the active site of DNMT1.[118] These inhibitors have also been proposed as potential antitumor drugs.[118]

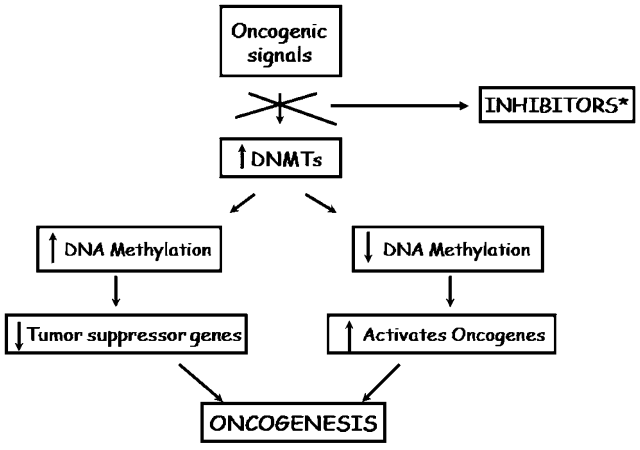

Figure 5 DNA methylation inhibitors. Successful inhibition of DNA methylation inhibitors (DNMTis) can prevent the suppression of tumor suppressor genes and continuous activation of proto-oncogenes.

Demethylation of aberrantly silenced genes can restore gene expression and function. Azacitidine (5-aza-C) and decitabine (5-aza-2′-deoxycytidine or 5-aza-2′-CdR) are two potent DNA demethylating agents. 5-aza-C was developed as a cancer chemotherapeutic agent and was thought to inhibit the enzymes that methylate the cytosine residues in DNA.[10,119] However, it is now considered that it demethylates methylated DNA and acts as an antitumor agent in many cancer cells.[120] Azacitidine is more toxic than its analog, decitabine.[22]

Decitabine has a short *in vivo* half-life and is able to reactivate previously silenced genes.[121,122] In clinical investigations in treating leukemia, decitabine was found to be most effective in an intensive dose with a short treatment time.[123] Decitabine is a potent cytotoxic agent and shows *in vitro* antitumor activity against breast cancer cells.[124] Both azacitidine and decitabine are effective in treating leukemia, which is characterized by hypermethylation.[35,125]

Zebularine, another potent demethylator, was found to be effective against cancer cells in many studies. In earlier studies, it was found to have toxic effects in cancer cell lines.[73] It has been shown to remove 25–60% of the methyl groups from methylated genes in a panel of seven human tumor cell lines.[94] The most promising features of the drug include its stability, low toxicity to normal cells, and that it can be taken orally.[126]

Administration of decitabine can reactivate silenced tumor suppressor genes and the histone deacetylase inhibitor, LAQ824 (LAQ), can activate genes related to cell cycle arrest.[123,127] These agents can synergistically produce greater antineoplastic effects on MDA-MB-231 breast cancer cells, thereby ensuring application of these agents for future clinical trials.[127] Lower doses of azacitidine and trichostatin A (TSA) are required to re-express ER in MDA-MB-231 (ER−) cells, when the drugs are used in combination than alone.[58] Thus, the combination of drugs, which directly affects DNA methylation with drugs, causing other epigenetic changes, has considerable potential in increasing therapeutic affects.

Cancer Prevention, Dietary Intake, and DNA Methylation

Nutrition influences susceptibility to cancer. Approximately 35–50% of all cancers have a dietary component in their etiology.[128] Some food constituents can promote the onset of cancer. Deficiency of fiber, folic acid, methionine/choline, zinc, selenium, and chemicals found only in fruits and vegetables all can also cause cancer.[129] Excess intake of alcohol, animal fats, and salt promote cancer.[130] It was predicted that the incidence of cancer in vegetarian subjects would be lower than in those on a meat diet. Even though the vegetarian diet has a lower intake of vitamin B and lower content of methionine, both of which are essential for eventual methylation, this does not cause cancer.[131]

The role of folic acid in cancer is still controversial. The methyl groups of 5-methylenetetrahydrofolate is the precursor of the methyl group of methionine and thereby of SAM.[132] Low intake of folate combined with high alcohol intake can result in global hypomethylation and cause colorectal cancer. An increased risk of breast cancer occurs if folic acid is not metabolized correctly and the resultant supply of methyl groups to DNA in premenopausal women is insufficient.[113,132]

Low intake of folate is associated with an increased risk of colorectal cancer.[113] *In vitro* studies have indicated that colon cancer cell lines, when deprived of folic acid, have decreased viability.[132] Uracil is misincorporated into DNA as a result of folate deficiency. However, once a cancerous lesion is present, folate intake enhances tumor growth.[133] Methionine is taken as a nutritional supplement in adulthood to correct some genetically based epigenetic defects.[29] However, excess intake of methionine can also impair DNA methylation. Nevertheless, folate deficiency, leading to aberrant methylation of DNA, is not the sole cause of colon carcinogenesis.

Caffeic acid and chlorogenic acid, two catechol containing coffee polyphenols, inhibit DNA methylation.[134] They increase the formation of *S*-adenosyl-L-homocysteine (SAH), an inhibitor of DNA methylation. Partial inhibition of methylation in the promoter region of the retinoic acid receptor beta (RAR-β) gene by both caffeic acid and chlorgenic acid was demonstrated in breast cancer cell lines MCF-7 and MDA-MB-231.[135]

DNA Methylation and Aging

The risk of cancer increases with age. Only 10% of children have a chance of getting cancer, whereas

adults have a 35% chance, because methylation of CpG islands in non-malignant tissues increases but the total number of methylated cysteine residues decreases with age.[81,136,137] Individual genes are progressively methylated during aging due to chromosomal instability.[138] Genes that change methylation status with age are tissue specific. The c-myc gene is hypomethylated in the spleen and the c-fox is hypermethylated in the liver but not in the spleen.[28]

The normal colonic mucosa of older females has higher methylation levels, making these cells more susceptible to differentiate into malignant cells.[103,139] Hypermethylation does not always result in malignancy. For example, hypermethylation of the estrogen receptor gene was observed in both normal and cancer colon tissues, suggesting that the relationship between hypermethylation and age in cancer might not be simple, and requires a more careful analysis.[8]

Concluding remarks

DNA methylation is important in gene regulation and expression. It is imperative to learn more about the regulation of how this simple and basic process becomes aberrant. The complete biological mechanisms that initiate and maintain methylation of DNA need to be fully explained. Both hypomethylation and hypermethylation of proto-oncogenes and/or tumor suppressor genes occurs in various cancers. Many known genes are aberrantly methylated in breast and colorectal cancers.

In studies with established inhibitors of DNA methylation and demethylation, some genes were shown to be able to resume normal function. Zebularine was shown to be effective, without many toxic affects, in clinical trials. Despite constant efforts, the most effective and least toxic drugs are yet to be discovered. Indeed, even more questions arise from the plethora of recent advances in our understanding of the underlying mechanisms of methylation in cancer and other malignancies.

Acknowledgements

This work was supported by the Claire Booth Luce Fellowship (to AA) and by the National Institutes of Health Grants R01HL070885 (to DKA) and R01HL073349 (to DKA) and Carpenter Chair (to RFM) of Creighton University.

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