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Epigenetic Events Associated with Breast Cancer and Their Prevention by Dietary Components Targeting the Epigenome

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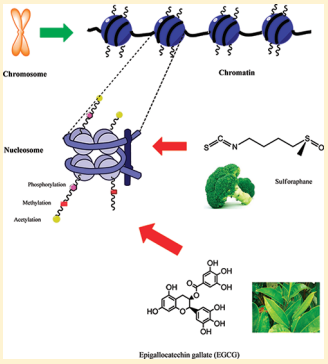
ABSTRACT: Aberrant epigenetic alterations in the genome such as DNA methylation and chromatin remodeling play a significant role in breast cancer development. Since epigenetic alterations are considered to be more easily reversible compared to genetic changes, epigenetic therapy is potentially very useful in reversing some of these defects. Methylation of CpG islands is an important component of the epigenetic code, and a number of genes become abnormally methylated in breast cancer patients. Currently, several epigenetic-based synthetic drugs that can reduce DNA hypermethylation and histone deacetylation are undergoing preclinical and clinical trials. However, these chemicals are generally very toxic and do not have gene specificity. Epidemiological studies have shown that Asian women are less prone to breast cancer due to their high consumption of soy food than the Caucasian women of western countries. Moreover, complementary/and or alternative medicines are commonly used by Asian populations which are rich in bioactive ingredients known to be chemopreventive against tumorigenesis in general. Examples of such agents include dietary polyphenols, (−)-epigallocatechin-3-gallate (EGCG) from green tea, genistein from soybean, isothiocyanates from plant foods, curcumin from turmeric, resveratrol from grapes, and sulforaphane from cruciferous vegetables. These bioactive components are able to modulate epigenetic events, and their epigenetic targets are known to be associated with breast cancer prevention and therapy. This approach could facilitate the discovery and development of novel drugs for the treatment of breast cancer. In this brief review, we will summarize the epigenetic events associated with breast cancer and the potential of some of these bioactive dietary components to modulate these events and thus afford new therapeutic or preventive approaches.



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INTRODUCTION

Breast cancer accounts for ∼30% of all cancers diagnosed in the United States, and it is the second leading cause of cancer death in women.[1] Overall, women have a >10% lifetime risk of developing invasive breast cancer.[2,3] Although extensive research has been done, the causes and mechanisms of breast cancer development and progression are complex and still remain inconclusive.[4,5] Mammary gland development initiates during fetal development and continues postnatally through puberty, pregnancy, lactation, and subsequent involution during which the gland undergoes extensive morphological and functional changes.[6−9] Moreover, development of cancer in the breast is envisioned as a stepwise process that begins in mammary ducts and progresses in four stages. In stage 0, the tumor is noninvasive, and growth of the cells does not affect the functions of neighboring cells and tissues. In stage 1, the tumor becomes invasive and begins to affect neighboring tissues but does not reach the lymph nodes. In stage 2, the cells in the individual lymph nodes begin clumping together and cause inflammation. In stage 3, the cancer spreads to other organs such as the brain, lung, or liver.[10,11] Although the mechanisms associated with breast cancer are genetic alterations, including specific gene amplifications, deletions, point mutations, chromosome rearrangements, and aneuploidy,[12] like other types of cancer, it is also driven by epigenetic mechanisms.[13−15]

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In the past few years, the mortality rate due to breast cancer has declined[10] probably due to early diagnosis by mammography screening, which initiates early treatment. However, the benefit of mammography is significant only for women over 40 years of age.[16,17] Although mammography is highly sensitive, it is not exclusively specific; sometimes, both false positive and negative results may be obtained. Therefore, for the better management of the disease a highly sensitive and specific diagnostic method for early detection of breast cancer in addition to mammography is necessary.[18] Within this context, a biochemical approach using blood/serum may be more appropriate and accurate for early detection of breast cancer. Currently, there is no ideal protein biomarker of breast cancer in plasma or serum that can be used with desired sensitivity and specificity.[19] Considering the benefits of epigenetics, DNA methylation change may be regarded as a useful biomarker of breast cancer[20−23] because compared to other sources such as mRNA and proteins, DNA is relatively stable, and it can be obtained as cell-free DNA from blood, ductal lavage fluids, nipple aspirate fluids as well as fine needle aspirates of the primary tumors.[19] A number of studies have reported the ability to detect breast cancer cells by epigenetic analysis in fine needle aspirations, nipple aspirates, and ductal lavages.[24−27] Therefore, epigenetic alterations such as specific gene promoter DNA methylation, histone acetylation/deacetylation, or other events hold promise as a tool for early detection of breast cancer.

Many of the risk factors proposed for breast cancer include individual genetic background, pregnancy, and lifestyle elements, such as diet and environment.[28] It was estimated that nearly one-third of all cancer deaths in the USA could be prevented through appropriate dietary modification.[29] Therefore, many studies have been focused on linking diet (such as a soy rich diet) with breast cancer prevention. However, the results are either inconclusive or remain uncertain.[30,31] Epidemiological studies indicate that women living in Asia have a lower breast cancer risk than women living in the western world, probably due to the difference in soy food product consumption.[30,32,33] However, genistein, a soy isoflavone, has been shown to stimulate the growth of MCF-7 cells (estrogen receptor positive breast cancer cells) *in vitro*, enhance the growth of mammary tumors in animal models, and also enhance the growth of breast tumors in ovariectomized athymic mice when administered with the diet.[34−36] Further studies indicate that childhood/adolescence exposure to soy provides protection against breast cancer later in life, whereas administration during adult life had no effect on mammary tumors.[37−39] Therefore, it is predicted that fetal and neonatal exposure to phytoestrogens may lead to epigenetic changes that protect them against the development of breast cancer in later life.[40]

Taken together, it is clear that epigenetic mechanisms play key roles in breast cancer development and are therefore potentially useful in prevention, prognosis, and perhaps treatment.[41,42] As epigenetic defects are reversible, attempts have been made to develop epigenetic drugs (epi-drugs) and to prevent breast cancer. Several synthetic epi-drugs such as 5-aza-2′-deoxycytidine (5-aza-DC, decitabine) and trichostine are currently available; however, they are very toxic and would be deemed nonspecific gene modulators.[24] In addition, several bioactive dietary components such as tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) have also been used as epi-drugs.[24] Several other bioactive dietary components and natural products have the potential to be used as preventive agents of epigenetic disorders observed in cancer models.[43−46] In this brief review, we will focus on major epigenetic events associated with breast cancer and discuss the potency of bioactive dietary components as epigenome modifiers, which may be used for the prevention of breast cancer.

EPIGENETICS, BREAST CANCER, AND EPIGENETIC DRUGS

Strictly speaking, the term epigenetics is defined as heritable changes in gene expression that do not involve DNA nucleotide sequences.[47−51] It is the "stable alterations in gene expression potential that arise during development and proliferation."[52] Furthermore, epigenetics is also defined as "the manifestation of a phenotype, which can be transmitted to the next generation of cells or individuals, without alteration of the DNA sequence (genotype)."[7] In general, epigenetics has been interpreted more widely to include any external effect on the phenotype (epigenator). Epigenetic therapy is a new and rapidly developing area and a potentially very useful form of therapy in breast cancer prevention due to the fact that epigenetic defects mostly occur at the chromosomal level in transformed cells and are thought to be more easily reversible in comparison with genetic defects.[53] At the biochemical level, the epigenetic alteration in chromatin conformation involves DNA methylation, several forms of histone modifications, and microRNA (miRNA) expression. These events modulate chromatin structure and in turn activate or silence gene expression.[15,54] The understanding of these epigenetic changes and their contributions to carcinogenesis is very important for further progress in the field of diagnosis, prognosis, and therapy of any cancer including breast cancer.

In the past few decades, molecular therapies for breast cancer have developed rapidly.[55] As per receptor status of the cancer cells, breast cancer can be divided into estrogen receptor positive (ER+), ER negative (ER−), and a basal like subtype that is triple negative for ER, the progesterone receptor (PR), and human epidermal growth factor 2 (HER2).[3] ER+ breast cancers are estrogen-dependent[3,56−58] and include luminal types A and B. ER− breast cancer is estrogen independent and includes subtypes in which HER2 (also known as ERBB2) is overexpressed. Approximately, 60% of the premenopausal and 75% of the postmenopausal women have estrogen-dependent breast cancer.[59,60] The selective estrogen receptor (ER) modulators (SERMs) such as tamoxifen, which competes with estrogen for binding to ER, selective ER down-regulators (SERDs) such as fulvestrant, which induces destabilization and degradation of ER, and aromatase inhibitors (AIs), such as anastrozole and letrozole, which reduce the production of estrogen in peripheral tissues and within the tumor by inhibiting aromatase enzyme activity, have become a standard therapy for estrogen-dependent breast cancer.[55,61] However, ER-negative cells are not responsive to estrogen; therefore, antiestrogenic drugs have no effect on ER− breast cancer.[56,62] A therapy that could reactivate ER expression in ER− cancer patients could reestablish cancer cell growth regulation through estrogen, and then, only antiestrogenic drugs could subsequently be used for treatment.[12] Investigations on MDA-MB-435 cells (ERα negative breast cancer cells) showed that ERα was induced in these cells by 5-aza-DC, a DNA methyltransferase (DNMT) inhibitor and a demethylating epigenetic drug used in the treatment of myelodysplastic syndrome.[56] Moreover, many histone deacetylase (HDAC) inhibitors can also reactivate ER expression in ER− cell lines.[63] Furthermore, there is increasing evidence which indicates that ER pathways involved in endocrine treatment are

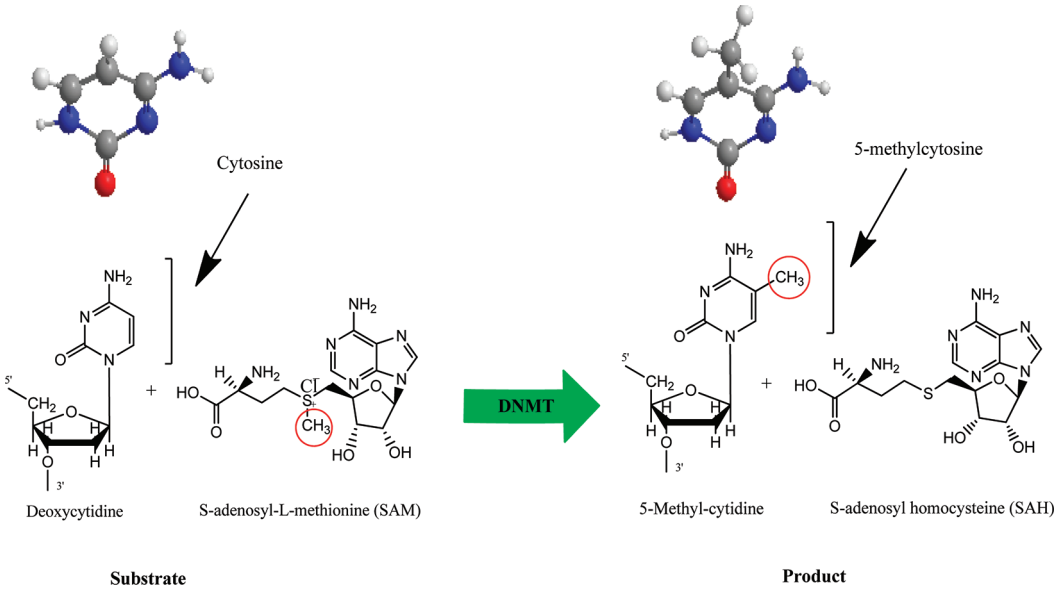

Figure 1. Schematic illustration of the enzymatic DNA methylation catalyzed by DNMTs using SAM as the methyl donor. After the donation of the methyl group (CH_3) SAM is converted to SAH. SAM = *S*-adenosyl-L-methionine; SAH = *S*-adenosyl-homocysteine. A sketch of a cytosine and its modified counterpart 5-methylcytosine is presented in the upper panel.

also under epigenetic regulation.[55] Therefore, targeting epigenetic mechanisms represents an active area for breast cancer drug development and therapy.

The number of genes that has been identified to be aberrantly methylated in breast cancer is rapidly growing. Studies indicate that in breast cancer, epigenetic alterations such as promoter hypermethylation and gene silencing occur in many genes participating in apoptosis (*HOXA5*, *RASSF1A*, *TWIST1*), DNA repair (*BRCA1*), metabolic events (*GSTP1*), tissue invasion, and metastatic processes (*CDH1*, *CDH13*).[13,26,55,64,65] These genes are not only hypermethylated in tumor cells but also showed increased epigenetic silencing in normal epithelium surrounding the tumor site, which is similar to cancerization.[66] A recent study identified 149 genes that are differentially expressed in ER+ versus ER− cells, differentially methylated on one or more promoter-proximal CpG islands, and exhibit an inverse correlation between CpG island methylation and mRNA abundance.[67] Moreover, many genes identified in breast cancer tissues which have hypermethylated CpG islands are located near the consensus sequences of the transcription factor binding sites,[68] which indicates further that in breast cancer the gene expression is also regulated by epigenetic mechanisms.

The drugs currently used in breast cancer patients to prevent epigenetic disorders (epi-drugs) are in the stage of preclinical and clinical trials.[15,55] The first epi-drug approved by US Food and Drug Administration (FDA) was the DNMT inhibiting nucleoside analogues 5-azacitidine and 5-aza-DC used for the treatment of myelodysplastic syndrome.[69,70] Since then, several nucleoside and nonnucleoside analogues for DNMT inhibitiors and histone deacetylase inhibitors have been described;[71] however, most of them are highly toxic and do not have gene specificity. Additionally, nonspecific demethylation has the risk of inducing the silencing of tumor-suppressor genes. [24]

The development of therapeutic strategies by using bioactive components from the diet to target breast cancer-dependent epigenetic mechanisms provides many advantages.[45,46] The dietary components are generally considered safe. They are readily available to most people because they are present in common food, and in contrast to many synthetic chemopreventive drugs, they have very low or no toxicity/side effects.[72] Moreover, some of these compounds have shown potential chemopreventive effects in a few clinical trials.[45,46] Therefore considerable interest is now emerging in the use of diet-derived botanicals/natural products for various cancer prevention and therapy approaches.[45,46,73,74] However, there are many disadvantages as well. For example, EGCG, the most potent bioactive chemical isolated from green tea and used in many cancer models as a chemopreventive agent, is very unstable under physiological conditions. Another compound curcumin, a yellow pigment present in the spice turmeric (*Curcuma longa*), is almost insoluble in water and is poorly absorbed from the gastrointestinal tract. [46,75,76] Despite these problems, more studies on bioactive dietary components as cancer preventive agents are warranted to take advantage of the potential offered by naturally occurring modulators.

DNA METHYLATION AND DNA METHYL TRANSFERASES

In mammals, DNA methylation is the most widely studied epigenetic modification which results in the addition of a methyl group to the carbon-5 (C5) position in the pyrimidine ring of cytosine located in the context of cytosine-phosphate-guanine (CpG) dinucleotide of the genomic DNA.[12] The reaction is catalyzed by the enzyme DNA methyltransferase (DNMT) with *S*-adenosyl-methionine (SAM) as the methyl donor (Figure 1).[43] In mammals, so far five members of the DNMT protein family have been discovered (DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L) of which only three were shown to possess catalytic methyltransferase activity (DNMT1, DNMT3A, and DNMT3B).[5,15,46] The mammalian DNMTs consist of two parts: the catalytic C-terminal domain, which is highly conserved among prokaryotes and eukaryotes, consists of 500 amino acids, and involved in cofactor binding and catalysis; and the regulatory N-terminal part consisting of 621 amino acids, required for

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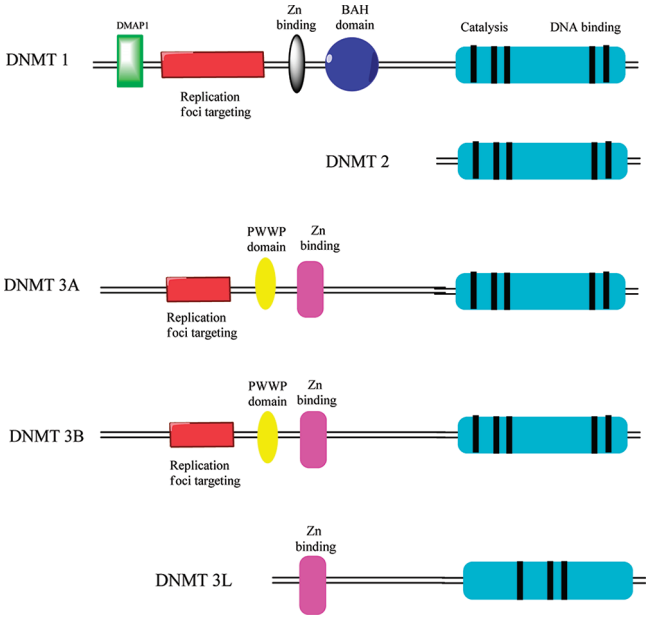

Figure 2. Schematic representation of the DNMT proteins. All DNMTs can be split into two main domains: regulatory and catalytic. The conserved regions in the catalytic domains are shown in black. The PWWP domain represents the prolin−tryptophan−tryptophan−proline motif found in DNMT3A and 3B. BAH domain = bromo-adjacent homology domain. Adapted with permission from ref 82. Copyright 2011 Elsevier.

discrimination between hemimethylated and unmethylated DNA and not essential for enzyme activities (Figure 2). The most abundant DNMT is DNMT1, which is a maintenance methyltransferase, positioned at the replication fork and maintains the methylation pattern during DNA replication. Human DNMT1 is located on chromosome 19p13.2 and consists of 1616 amino acids. The DNMT2 is the smallest among the DNMTs (391 amino acids), comprises mainly the C-terminal domain, and has no regulatory N-terminal domain. This enzyme participates in the recognition of DNA damage, DNA recombination, and mutation repair.[77] Moreover, it was also shown to methylate $tRNA^{Asp}$,[78,79] which suggested that the substrate specificity of DNMT2 might be different from that of other DNMTs. DNMT3A and DNMT3B are *de novo* methyltransferases upregulated in aging cells and have a C-terminal catalytic domain similar to that in DNMT1. The amino acid identity between DNMT3A and DNMT3L is very high, but the latter (DNMT3L) lacks any catalytic activity due to the absence of conserved catalytic residues (Figure 2). However, DNMT3L is required for the catalytic activity of both DNMT3A and DNMT3B. In breast cancer, DNMT3B mRNA was found to be overexpressed.[80,81] The crystal structure of all DNMTs is now available, but the information on DNMT1 is very limited.[82] In breast cancer patients, the mean level of DNMT1, DNMT3A, and DNMT3B overexpression ranged from 1.8- to 2.9-fold, and probably, DNMT3B played the predominant role.[5]

The mechanisms by which DNMT methylates cytosine C5 has been studied extensively (Figure 1).[43,82−85] Briefly, DNMT forms a complex with DNA, and the cytosine which will be methylated flips out of the DNA double helix. The thiol of the cysteine residue in the active sites of the DNMTs acts as a nucleophile that attacks the 6-position of the target cytosine to generate a covalent DNA−protein intermediate. The intermediate then accepts a methyl group of the methyl-donating cofactor SAM to form the 5-methyl covalent adduct and *S*-adenosyl-L-homocysteine (SAH). After methyl transfer, the proton at the 5 position is attracted by a basic residue in the active site of the enzyme, which is removed from the 6-position by β-elimination to generate the methylated cytosine and free enzyme. The 5-methylated cytosine base then flips back into its original position within the DNA.[43,82] DNMTs can also silence genes by other mechanisms. The formation of a repressive transcription complex by histone deacetylases (HDACs) and the methyl-CpG binding domain (MBD) family of proteins at the promoter regions does not require promoter methylation.[54,86] Moreover, DNMT1 through its N-terminus binds to HDAC2 and a DNMT1-associated protein called DMAP1 to form a complex at the replication fork.[87] This complex converts acetylated histones to the deacetylated inactive form and highlights the interplay between methylation and acetylation processes in epigenetic regulation.[54]

DNA methylation of the promoter region of a gene has been shown to be an important factor in its ability to bind different transcription factors.[88,89] CpG dinucleotide rich regions located in the 5′-end region of the genes are called CpG islands.[90,91] In humans, approximately 60% of the genes have CpG islands.[5] Methylation of CpG islands is the most commonly studied epigenetic change in human cancers.[92] Most CpG islands are unmethylated in noncancerous cells, which promote active gene transcription. In cancer cells, CpG islands become hypermethylated, leading to the inactivation of tumor suppressor genes. For example, well-known tumor suppressor genes like *p16INK4a*, *APC*, and *BRCA1* genes are silenced in breast tumors due to DNA hypermethylation.[93−95] However, some genes are activated during carcinogenesis due to hypomethylation. In breast cancer, the overexpression of the γ synuclein gene (SNCG) occurs due to SNCG promoter hypomethylation.[96]

There are many bioactive dietary compounds that can modulate the DNMT enzyme activities and thus promoter methylation pattern of genes in breast cancer (Figure 3; Table 1).[45,46] Apigenin in Parsley (*Petroselinum*), curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in turmeric (*Curcuma longa*), EGCG in green tea (*Camellia sinensis*), genistein in soybean (*Glycine max*), resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) in red grapes (*Vitis vinifera*), sulforaphane (SFN) in cruciferous vegetables (*Brassicacaae*), and the caffeic acid and chlorogenic acid as coffee polyphenols are reported to inhibit DNMT enzyme activity in various cancer/breast cancer models.[46,97] Several dietary components such as baicalein, myricetin, protocatechuic acid, phloretin, sinapic acid, resveratrol, rosmarinic acid, ellagic acid, betanin, cyanidin, and galangin have been studied for potential effects on the activity and expression of DNMTs in human breast cancer MCF-7 cell lines.[98] Among these 12 compounds, betanin was found to be the weakest, and rosmarinic and ellagic acids were the strongest inhibitor of DNMT enzymes. But the methylation or the expression of *RASSF1A*, *GSTP1*, or *HIN1* genes in MCF-7 cells remained unaltered by these dietary polyphenols. However, decitabine partially demethylated and reactivated these genes in MCF-7 breast cancer cells.[98] EGCG can also reduce DNMT enzyme activities indirectly by reducing *S*-adenosyl-L-methionine (SAM) and increasing *S*-adenosyl homocysteine (SAH) and homocysteine levels.[99] In MCF-7 and MDA-MB-231 breast cancer cell lines, EGCG inhibited DNMT and partially demethylated retinoic acid receptor β2 (*RAR*β*2*), which exists in a hypermethylated state in these cells.[99]

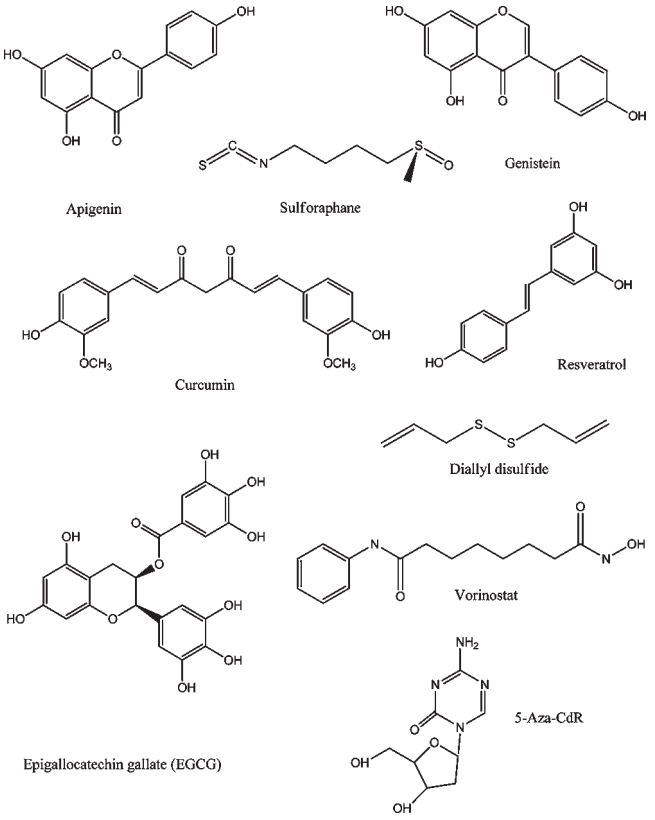

Figure 3. Chemical structure of representative bioactive dietary components, DNMT inhibitor 5-aza-2′-deoxycytidine (5-aza-CdR), and HDAC inhibitor SAHA (vorinostat).

A novel pro-drug of EGCG (pEGCG) and SFN significantly inhibited DNMT activities in MCF-7 and MDA-MB-231 cells and down-regulated *hTERT* expression.[46,100] DNMT1, DNMT3A, and DNMT3B proteins were downregulated in both MCF-10 AT benign cells and MCF-7 breast cancer cells by genistein.[28] Further, genistein and lycopene (a carotenoid derived from tomato) partially demethylated the *GSTP1* but not *RARβ2* gene in MDA-MB-468 (ER−) human breast cancer cells.[101] However, an *in vivo* study indicated that genistein hypermethylated *RARβ2* and *CCND2* genes in the intraductal tissue of premenopausal women.[102] Resveratrol has been shown to have weaker DNMT inhibitory activity than other dietary components such as EGCG.[46] It was able to inhibit DNMT1 enzyme activity in ER-α expressing MCF-7 cells significantly but not in MDA-MB-231 cells. Moreover, the promoter methylation pattern on the *RARβ2* gene remained unaltered by resveratrol in both cell lines (MCF-7 and MDA-MB-231).[103] Furthermore, in a study with MCF-7 cells, it was demonstrated that resveratrol is able to prevent epigenetic silencing of the *BRCA-1* gene induced by the aromatic hydrocarbon receptor (AHR).[104] Two common coffee polyphenols, caffeic acid and chlorogenic acid, partially inhibited the methylation of the *RARβ* promoter in MCF-7 and MDA-MB-468 cells.[97]

The mechanisms of inhibition of the DNMT enzyme activity by these bioactive compounds in breast cancer models was also studied by many investigators. The classical synthetic inhibitor 5-aza-DC, a cytidine analogue, inhibits the DNMT enzyme by forming irreversible covalent bonds with DNMT1 and incorporating itself into the replicating DNA, which induces the degradation of DNMT1.[24,105] The non-nucleoside DNA methylation inhibitors (such as procainamide) demethylate DNA by binding to GC-rich DNA sequences.[106,107] Bioactive natural products such as quercetin, fisetin, and myricetin which have catechol structures are readily methylated by SAM in the presence of catechol *O*-methyltransferase (COMT), resulting in the conversion of SAM to SAH.[97,99] By using molecular modeling techniques, it was demonstrated that EGCG, a non-nucleoside analogue and a competitive inhibitor of DNMT1, exerts its inhibitory effect by blocking the entry of the key nucleotide cytosine into its active site by hydrogen bonds. Mg^{2+} can enhance the reaction.[108] Other tea polyphenols, such as (−)-epicatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin have also been implicated in DNA methylation inhibition; however, they are not as potent as EGCG.[45] The coffee polyphenols, caffeic acid and chlorogenic acid, inhibit DNA methylation indirectly (noncompetitive) by forming SAH, as a consequence of COMT-mediated O-methylation.[99] Genistein showed both competitive and noncompetitive inhibition of DNMT activity.[109] Molecular docking analysis of the interaction between curcumin and DNMT1 suggested that curcumin covalently blocks the catalytic thiolate of C1226 of DNMT1 to exert its inhibitory effect.[110,111] The inhibition by curcumin seems to be comparatively lower than that of other bioactive dietary components such as EGCG and genistein.[110−112]

HISTONE MODIFICATION

Another prevalent epigenetic mechanism influencing gene transcription is histone modification. Much evidence of the cross-talk between DNA methylation and histone modifications have been reported.[113−115] Histone proteins are responsible for maintaining chromatin structure either in an accessible or inaccessible state to various transcriptional activators and repressors for their binding to gene promoters.[116] The nucleosomes of euchromatic DNA contain an octamer of four core histones (H2A, H2B, H3, and H4) around which 146 bp of DNA are wrapped.[117−121] Histones can be modified post-translationally by acetylation, methylation, phosphorylation, sumoylation, ubiquitination, ADP ribosylation, deamination, proline isomerization, and newly identified propionylation,[110,122,123] and all these effects can alter the accessibility of DNA to transcriptional activity. Most of these modifications are reversible.[120] Moreover, gene repression or activation by histone acetylation or methylation is dependent on the specific residues. The most important histone modifications, having effects on gene expression, are located on histone H3 and histone H4.[124] One of them, known to have a gene silencing role and to have a strong relationship with DNA methylation, is the di or trimethylation of lysine 9 of histone 3 (H3K9me2 or H3K9me3). But methylation on the same histone on lysine 4 (H3K4me) is related to gene activation.[125,126] All these modifications are catalyzed by specific enzymes.[15]

Acetylation of histones occurs within the amino-terminal tails protruding from the surface of the nucleosomes and also on the globular core region by transfer of acetyl group from acetyl-CoA to the ε amino group of lysine residues (N^{ε}). The reaction is catalyzed by histone acetyl transferase (HAT). The global acetylation of histone tails decreases electrostatic interactions between the negatively charged DNA and the basic lysine residues.[127] As a result, the chromatin becomes more open to provide access for transcription factors to DNA. Conversely, deacetylation,

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Table 1. Effects of Bioactive Dietary Components on Epigenetic Regulation in Breast Cancer Models[a]

	epigenetic effects/cell lines			regulated genes/cell lines
name of the compd	DNA methylation	histone modification	microRNA (miRNA) interference	
(−)-epigallocatechin-3-gallate (EGCG)	partially inhibited the methylation status of the promoter region of *RARβ*/MCF-7; MDA-MB-231[99b]	decreased acetyl-H3, acetyl-H3K9 and acetyl-H4 levels in *hTERT* promoter/MCF-7, MDA-MB-231[100]		*hTERT*/ MCF-7, MDA-MB-231[99,100]
proEGCG		decreased acetyl-H3, acetyl-H3K9 and acetyl-H4 levels in *hTERT* promoter/MCF-7, MDA-MB-231[100]		*hTERT*/MCF-7, MDA-MB-231[100]
caffeic acid	partially inhibited the methylation of *RARβ* promoter/MCF-7, MDA-MB-231[97]			*RARβ*/MCF-7, MDA-MB-231[97]
chlorogenic acid	partially inhibited the methylation of *RARβ* promoter/MCF-7, MDA-MB-231[97]			*RARβ*/MCF-7, MDA-MB-231[97]
genistein	down regulates the expression of *DNMT1*, *DNMT3A*,*DNMT3B*/MCF-7;[28] down regulates expression of *DNMT3A*/MCF-10AT;[28] hypo-methylation in hTERT promoter/MCF-7[28] partially demethylates *GSTP1* promoter/ MDA-MB-468[101] induced dose- specific changes in *RARβ2* and *CCND2* methylation/human intraductal tissue(mammary gland of premenopausal women)[102]	inactivated histone trimethyl-H3K9/ MCF-7[28] reduced basal expression of acetylated histone 3 (H3)/MCF-7[40]		DNMT1, DNMT3A, DNMT3B, hTERT/ MCF-7[28] *RARβ2, CCND2*/ human intraductal tissue[102]
resveratrol	inhibted DNMT enzyme activity/MCF-7 [98]	inhibited BRCA1 mutant tumor growth through activating SIRT1/ Mouse BRCA1 mutant and wild type cell lines[155]		
	modulate the recruitment of MBD2 to the BRCA-1 promoter/MCF-7[104]	induced SIRT1/MCF-7[156]		
sulforaphane	decreased DNMT1 and DNMT3A/ MCF-7 and MDA-MB-231[46]	hyperacetylation/MCF-7 and MDA-MB-231 inhibited HDAC activity/MCF-7, MDA-MB-231, MDA-MB-468, T47D[154]		*hTERT*/MCF-7, MDA-MB-231 [46]
baicalein	inhibted DNMT enzyme activity/MCF-7[98]			
myricetin	inhibited DNMT enzyme activity/MCF-7[98]			
protocatechuric acid	inhibited DNMT enzyme activity/MCF-7[98]			
phloretin	inhibited DNMT enzyme activity/MCF-7[98]			
sinapic acid	inhibited DNMT enzyme activity/MCF-7[98]			
rosmarinic acid	inhibited DNMT enzyme activity and DNMT1 protein; increased DNMT1 mRNA/MCF-7[98]			
ellagic acid	inhibited DNMT enzyme activity/MCF-7[98]			
betanin	inhibited DNMT enzyme activity/MCF-7[98]			
cyanidin	inhibited DNMT enzyme activity/MCF-7[98]			
galangin	inhibited DNMT enzyme activity/MCF-7[98]			
syringic acid	inhibited DNMT enzyme activity/MCF-7[98]			
lycopene	demethylation of *RARβ2* and *HIN1*/MCF10A; partially demethylates *GSTP1*/MDA-MB-468[101]			*GSTP1*/MDA-MB-468[101]
S-allylmercapto cysteine		induced histone acetylation/T47D[147]		
curcumin			upregulated miR-15a and miR-16/MCF-7[178]	

[a] *BRCA1*, breast cancer associated gene-1; *CCND2*, cyclin D2; *DNMT*, DNA methyl transferase; *hTERT*, human telomerase reverse transcriptase; *GSTP1*, glutathione-*S*-transferase Pi 1; *HIN-1*, high in normal 1; sirtulin 1; RAR*β*, retinoic acid receptor *β*; miR-15a, MicroRNA-15a. Cell lines, MCF-7 (estrogen receptor positive human breast cancer cell lines); MDA-MB-231 (estrogen receptor negative human breast cancer cell lines); MDA-MB-468 (estrogen receptor negative human breast cancer cell lines); MCF-10A and MCF-10AT (non-tumorogenic human breast epithelial cell lines); T47D, human ductal breast epithelial estrogen receptor positive tumor cell line. [b] Superscript numbers correspond to the original article cited in the text and arranged in the reference list.

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which is catalyzed by histone deacetylases (HDACs), removes the neutralizing acetyl charge and induces chromatin condensation and gene inactivation and silencing (Figure 4).[114] HATs also acetylate several nonhistone proteins such as GATA1, E2F1, pRB, or TP53 frequently associated with cellular transformation.[128] HATs cannot bind to target gene promoters directly but rather are recruited by DNA-bound transcription factors;[129] however, HDACs whose primary role is to oppose the activity of HATs are recruited to the hypermethylated CpG islands of tumor suppressor genes via MBDs or independently of methylation by specific transcription factors.[130] Many nonhistone proteins are also used as substrates for HDACs.[130−133] HATs can be classified into five families: the GNAT family, the MYST family, the p300/CBP family, the SRC family, and the TAFII250 family.[43,129] Until now, 18 human HDACs have been identified and are grouped into four classes, Classes I, II, III, and IV, based on their homology with yeast HDACs and their phylogenetic conservation.[134] Histone methylation occurs in lysine and arginine residues, catalyzed by histone lysine methyl transferases (lysine) or histone arginine methyl transferases (arginine).[135] At present, there are 24 known sites of methylation of histones: 17 are lysine residues, and 7 are argine residues.[136] Furthermore, lysine side chains may be mono-, di-, or trimethylated, whereas the arginine side chain may be either mono- or dimethylated. In contrast to histone acetylation, histone methylation does not change histone's charge.[137] Recruitment of specific motif bearing factors to the methylated lysine is responsible for affecting chromatin structure. Histone demethylation is catalyzed by histone demethylases.[138,139]

HAT and HDAC inhibitors could potentially represent new treatment options for cancer.[42,140−142] Therefore, considerable attention has been paid to bioactive dietary components as breast cancer preventive agents targeting HAT and HDAC activities. Although direct cancer prevention by specific modulators of HAT has not yet been demonstrated,[44] several natural products, such as anacardic acid found in cashew (*Anacardium occidentale*), garcinol, a polyisoprenylated benzophenone derivative from *Garcinia indica*, and curcumin from turmeric, have the potential to inhibit p300 and p300/CBP associated factor (PCAF) activity.[111,143−145] EGCG has shown to specifically inhibit HAT but not HDAC in B lymphocytes.[146] *S*-allylmercaptocysteine, a compound found in garlic (*Allium sativum*), is able to induce histone acetylation in T47D human breast cancer cells.[147] Further studies are necessary to evaluate the potency of these bioactive dietary compounds as HAT inhibitors in breast cancer models.

Currently, a large number of structurally diverse HDAC inhibitors have been synthetically developed or purified from natural sources.[15,46] Among them suberoylanilide hydroxamic acid (SAHA or vorinostat) has been approved for treatment of cutaneous T-cell lymphomas.[71,148] HDAC inhibitors are classified into four main groups: hydroxamic acids, cyclic peptides, aliphatic acids, and benzamides.[134,137] Moreover, it was also shown that the HDAC inhibitors played different roles in ER+ and ER− breast cancer cells (Table 1).[149] The tea polyphenol EGCG, when applied to MCF-7 cells (ER+), can decrease *hTERT* promoter methylation and ablate histone H3Lys9 acetylation.[150] In MDA-MB-231 cells (ER−), EGCG together with trichostatin A (TSA) can reactivate ERα expression via remodeling of the chromatin structure of the ERα promoter by altering the status of histone acetylation and methylation. A decrease in binding of the transcription repressor complex, Rb/p130-E2F 4/5-HDAC1-

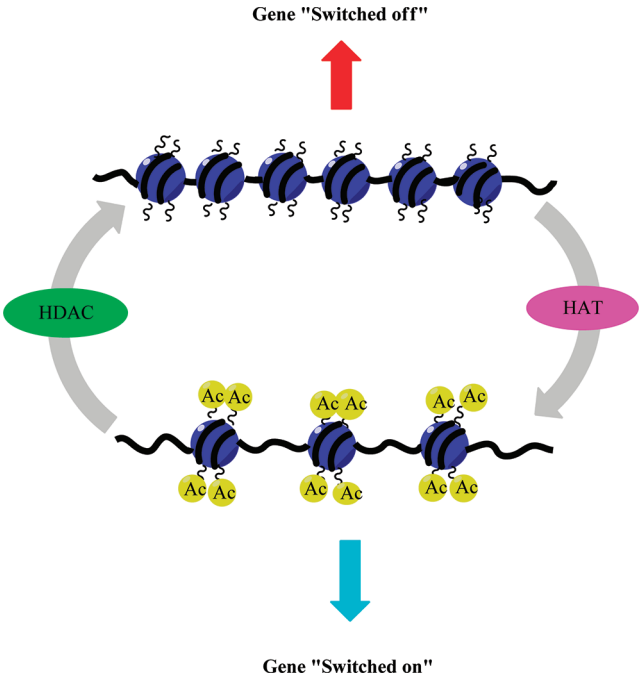

Figure 4. Schematic representation of histone modifications (acetylation and deacetylation). HATs induce relaxed chromatin which allows access to the various transcriptional factors associated with gene activation (gene switched on). HDACs induce closed chromatin associated with gene repression (gene switched off). HAT = histone acetyltransferase; HDAC = histone deacetylases; AC = acetylation.

SUV39H1-DNMT1, into the regulatory region of ERα promoter by EGCG and TSA was also observed.[151] Sulforaphane (SFN) is an isothiocyanate, derived from glucoraphanin in broccoli and broccoli sprouts.[46] Like other isothiocyanates, SFN is metabolized via the mercapturic pathway.[152] A phase I trial of broccoli sprout extracts containing glucosinolates and isothiocyanates produced no adverse effects on human volunteers.[153] Computer modeling predicted that SFN-cysteine (SFN-Cys) was a good fit for the HDAC active site.[152] Further studies indicate that SFN is able to inhibit HDAC activity in human breast cancer cell lines (MDA-MB-231, MDA-MB-468, MCF-7, and T47D) without altering the acetylation of H3 or H4.[154] In another study, it was documented that SFN inhibited HDAC activity in MCF-7 and MDA-MB-231 human breast cancer cells and increased the level of active chromatin markers such as acetyl-H3, acetyl-H3K9, and acetyl-H4, whereas it decreased the inactive chromatin markers like trimethyl-H3K9 and trimethyl-H3K27 and remodeled the chromatin structure of *hTERT* promoter (Table 1).[46] However, EGCG and pEGCG decreased the level of acetyl H3, acetyl-H3K9, and acetyl-H4 to the *hTERT* promoter in MCF-7 and MDA-MB-231 cells.[100] Genistein inactivated histone trimethyl-H3K9 followed by transcriptional repression of *hTERT* in human breast cancer cells.[28] Long-term genistein treatment (40−60 days) inhibited the growth of MCF-7 cells and markedly reduced the basal expression of acetylated histone 3 (H3).[40] *S*-Allylmercaptocysteine, a metabolite of diallyl disulfide, which is found in garlic and other *Allium* species, has been shown to induce histone acetylation in T47D human breast cancer cells.[147] Resveratrol, a phytoalexin from grape, inhibited the growth of BRCA 1 mutant tumor cells by upregulating SIRT1 (the mammalian homologue of yeast sir2 belongs to type III HDAC).[155] Treatment of breast

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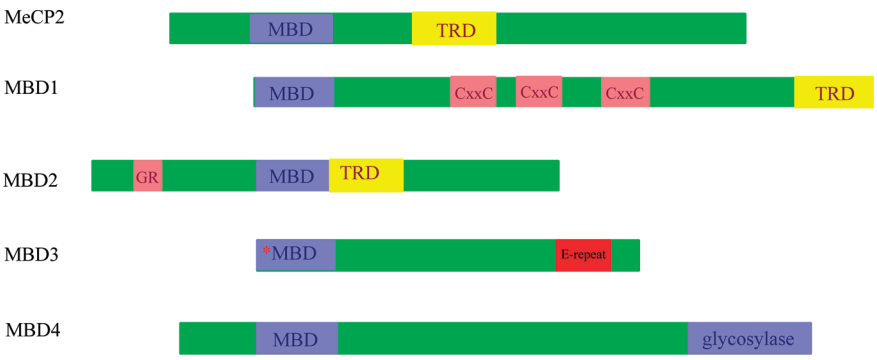

Figure 5. Schematic representation of methyl-CpG binding proteins (MBD) found in humans. The primary amino acid sequence of the MBD motif is conserved among the various members of the family. MBD = methyl CpG binding domain; MeCP2 = methyl-CpG-binding protein 2; MBD1 = methyl-CpG-binding domain protein 1; MBD2 = methyl-CpG binding domain protein 2; MBD3 = methyl-CpG-binding protein 3; MBD4 = metnyl-CpG binding domain protein 4; TRD = transcription repression domain present in MeCP2, MBD1, and MBD2. CxxC = cysteine rich domain (binds unmethylated DNA). GR = glycine and arginine residues; E-repeat = glutamate repeat. Modified from ref 163.

cancer cells with resveratrol induces SIRT1 (a member of the sirtuin protein family of NAD^+-dependent deacetylase) deacetylase activity, which promotes SIRT1 association with p300 (an HAT) and down-regulates p300 transferase activity that impairs the β-catenin and NFκB-p65 signaling cascades in MCF-7 cells.[156] All 12 dietary polyphenols evaluated in MCF-7 cells for DNA methylation and gene activation analysis by Paluszczak et al.[98] were unable to alter the global methylation pattern of histone H3.

METHYL-CPG-BINDING DOMAIN PROTEINS

Along with DNMTs, the methyl-CpG-binding domain (MBD) family of proteins plays an important role in epigenetic silencing of tumor suppressor genes. These proteins bind specifically to a methylated gene and mediate transcriptional repression by altering chromatin structure.[157,158] It has been proposed that MBD proteins serve as the bridge between histone modification enzymes and hypermethylated DNA associated with gene activation.[159] MBDs recruit histone-modifying complexes containing histone deacetylases (HDACs) and histone methyltransferases (HMTase) to methylated sites resulting in an active chromatin configuration around the genes.[160] Until now, five MBD genes have been identified in mammalian cells that encode MeCP2, MBD1, MBD2, MBD3, and MBD4 proteins (Figure 5). They all share a methyl-binding domain that allows them to bind to hypermethylated promoters of CpG islands of tumor suppressor genes and mediate the interaction between histone modification and methylation.[157,158,161,162] MBD4, which is primarily a thymine glycosylase, is involved in DNA repair.[159] In mammals, MBD3 is unable to bind methylated CpGs due to a mutation in the MBD.[163] Human MBD genes are considered as house keeping genes because they are widely expressed in somatic tissues.[158] In breast cancer, MeCP2 is overexpressed and appears to be associated with ER positivity.[164] MBD2 has also been reported to be involved in the repression of GSTP1 transcription in MCF-7 breast cancer cells.[165] Polymorphism in MBD2 is associated with reduced risk of breast cancer among premenopausal women.[166] In MCF-7 and MDA-MB-231 breast cancer cells, MBDs were identified in hypermethylated gene promoters.[167] Although MBD proteins play an important role in epigenetic modification, studies on targeting MBD proteins by bioactive dietary components as preventing agents for breast cancer have been very limited. Resveratrol was shown to modulate the recruitment of MBD2 to the BRCA-1 promoter in MCF-7 breast cancer cells (Table 1).[104]

MICRORNA

The most recently emerged participant in the epigenetic field is a family of small regulatory RNAs called microRNAs (miRNA), which are small noncoding RNAs ranging in size from 19 to 24 nucleotides that inhibit protein expression of target genes.[168] These RNAs regulate the expression of target genes by sequence-specific binding to the 3′-untranslated region (3′UTR) of target mRNAs, resulting in either mRNA degradation or inhibition of mRNA translation.[169] miRNA can directly or indirectly regulate cancer progression either by acting as the tumor suppressor or by altering epigenetic modifying enzymes.[46] Extensive analyses of genomic sequences of miRNA genes have shown that approximately half of them are associated with CpG islands.[170,171] Current bioinformatics tools predict that each miRNA recognizes an average of 100−200 different mRNA targets.[168] Epigenetic silencing of miRNA may be a reflection of tissue specificity. Upregulation of miR-205 is seen in lung, bladder, and pancreatic cancers; however, down-regulation is seen in prostate, breast, and esophageal squamous cell carcinoma.[172] Most importantly, expression patterns of miRNAs were correlated with tumor stage, proliferation index, estrogen and progesterone receptor expression, and vascular invasion.[173] Therefore, miRNAs can be used as a biomarker of cancer progression and development. In breast cancer, epigenetic silencing of several miRNAs is a frequent and early event.[5,171,174] Many of the miRNAs including miR-126, miR- 9-1, miR-10b, miR-125b, miR-145, miR-21, and miR-155 are reported to be deregulated in breast cancer.[171,173,175] The differentiation program of epithelial to mesenchymal transition (EMT)[176] involves changes in a number of miRNAs.[177] Some of these miRNAs have been shown to control cellular plasticity through the suppression of genes involved in defining the epithelial and mesenchymal cell states.

Although miRNAs are sensitive to chemical compounds, including the DNA-demethylating agent 5-aza-CD, studies with bioactive dietary components and their role in miRNA expression and functions in breast cancer are very scarce. One study showed that expression of miR-15a and miR-16 was upregulated in curcumin-treated MCF-7 cells (Table 1) and that upregulation can reduce the expression of Bcl-2.[178] In other breast cancer cell lines such as SKBR-3 and Bcap-37, curcumin was also able to increase the expression of miR-15a and miR-16.[178]

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CONCLUSIONS AND FUTURE DIRECTIONS

In the past few decades, significant efforts have been made toward understanding the molecular mechanisms of breast cancer development and the therapeutic potential of many synthetic drugs and natural products in prevention of this disease. The reversibility of epigenetic alterations, their prevalence in the cancer genome, and their significant role in tumor biology have attracted the attention of many investigators to develop epigenome altering anticancer drugs or epi-drugs[179] and their use for breast cancer prevention. Although the search for epigenetic-based markers in breast cancer has come a long way in recent years, no single identified marker has made the transition into the clinic.[112] As mentioned earlier, the first successful epi-drugs were the two demethylating azanucleosides, 5-azacytidine and 5-aza-2CD, which inhibit DNA methylation and turn on the silenced genes after incorporating them into the DNA of the dividing cells. There are other demethylating epi-drugs which can block the catalytic/cofactor-binding sites of DNMTs or can target their regulatory mRNA sequences; however, the efficacy of these epi-drugs is very limited. Attempts have also been made to target other epigenetic events such as histone modification which led to the development of a series of HDAC inhibitors from a heterogeneous group of chemical compounds which can interact mostly with the catalytic pocket of HDACs. Although the miRNAs have the great potential to be used in diagnostic, progonosis, and therapy of breast cancer, relatively little information is available about the epigenetic regulation of miRNA genes. Improved understanding of miRNA epigenetics may lead to the development of a new generation of drugs that can be used for the prevention of breast cancer

From this and earlier reviews by others,[45,46] it is evident that several dietary components have the potential to target many of the epigenetic events and can be used as epi-drugs for breast cancer prevention. Although we have mainly focused on breast cancer, these bioactive compounds can be effective as epi-drugs in other cancers. But our understanding of the action of currently known epigenetic modifiers is far from complete; therefore, the same compounds may show different activities in different tissue models. In addition, most of the data discussed in this review are based on the studies in cancer cell lines, with limited *in vivo* corroboration. Even data obtained through primary tumor cell cultures are inadequate to establish unambiguous roles in breast cancer development. For example, cell lines do not go through the complete cycle of differentiation and involution that are observed in mammary epithelial cells *in vivo*. Moreover, epigenetic changes are reversible, and therefore, prolonged treatment with epigenetic drugs, no matter whether synthetic or natural, is necessary. Thus, discontinuation of the drugs has the possibility to reactivate tumor growth. In addition, epigenetic drugs may not be free of adverse effects. The consumption of excessive amounts of bioactive dietary supplements may disrupt the rhythms of epigenetic events which are not desirable, and such treatments could lead to a decrease in quality of the life. Often issues of bioavailability, metabolism, and pharmacokinetics may also limit the effects of these dietary components. Moreover, the effect of normal dietary consumption of a single compound may often be insignificant without adequate dosing. Therefore, there is an urgent need to identify optimal indications, doses, dose regimens, and durations of therapy with these bioactive dietary components for future clinical trials in breast cancer. Moreover, the combination of these compounds or the additive effect of different dietary chemicals may enhance effects. Therefore, a better understanding of the interrelationships among dietary components, epigenetic modifications, and breast cancer is necessary. More probing research is needed to identify and characterize the function of these and other bioactive food components on specific epigenetic events and to determine the utility of interventions with these components for breast cancer prevention.

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ABBREVIATIONS

COMT, catechol *O*-methyltransferase; DNMT, DNA methyltransferase; 5-aza, 5-azacytidine; 5-aza DC, decitabine; EGCG, (−)-epigallocatechin-3-gallate; HAT, histone acetyl transferase; HDAC, histone deacetylases; HER2, human epidermal growth factor receptor 2; MBD, methyl-CpG binding domain; miRNA, microRNA; SERM, selective estrogen receptor modulator; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl-L-methyonine; SERD, selective estrogen receptor down regulator; SFN, sulforaphane; SNCG, γ synuclein gene; TSA, trichostatin A

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