

**Minireview**

# Targeting the Epigenome with Bioactive Food Components for Cancer Prevention

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## Key Words

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## Abstract

Epigenetic processes participate in cancer development and likely influence cancer prevention. Global DNA hypomethylation, gene promoter hypermethylation and aberrant histone post-translational modifications are hallmarks of neoplastic cells which have been associated with genomic instability and altered gene expression. Because epigenetic deregulation occurs early in carcinogenesis and is potentially reversible, intervention strategies targeting the epigenome have been proposed for cancer prevention. Bioactive food components (BFCs) with anticancer potential, including folate, polyphenols, selenium, retinoids, fatty acids, isothiocyanates and allyl compounds, influence DNA methylation and histone modification processes. Such activities have been shown to affect the expression of genes involved in cell proliferation, death and differentiation that are frequently altered in cancer. Although the epigenome represents a promising target for cancer prevention with BFCs, few studies have addressed the influence of dietary components on these mechanisms *in vivo*, particularly on the phenotype of humans, and thus the exact mechanisms whereby diet mediates an effect on cancer prevention remains unclear. Primary factors that should be elucidated include the effective doses and dose timing of BFCs to attain epigenetic effects. Because diet-epigenome interactions are likely to occur *in utero*, the impact of early-life nutrition on cancer risk programming should be further investigated.

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## Introduction

Cancer is a leading cause of death worldwide and the number of cases and deaths are expected to more than double in the next 20–40 years [1]. According to current estimates, the disease is 30–40% preventable over time with appropriate food and nutrition, regular physical activity and avoidance of obesity [2]. Several bioactive food components (BFCs) have been identified that have cancer prevention potential. Among them, methyl-group donors, polyphenols, selenium, retinoids, fatty acids, isothiocyanates and allyl compounds can be highlighted [3]. These BFCs affect different stages of carcinogenesis by modulating biological processes such as carcinogen detoxification, DNA repair, oxidative stress, inflammation and cell proliferation, and death and differentiation. At the genomic level, certain BFCs interfere with multiple cell signaling networks by direct or indirect activation of transcription factors [4, 5]. In recent years, the epigenome has gained much attention as a promising molecular target for cancer prevention with BFCs [6–10].

Epigenetics is defined as heritable changes in gene expression that are not accompanied by alterations in DNA sequence [11]. The main epigenetic processes are DNA methylation, histone modifications, including acetylation and methylation, and chromatin remodeling. They participate in diverse regulatory processes in the cell, including gene and microRNA expression, DNA-protein interactions, suppression of transposable element mobility, cellular differentiation, embryogenesis, X-chromosome inactivation and genomic imprinting [12, 13]. While the term ‘epigenetics’ typically refers to the analysis of a limited number of loci, ‘epigenomics’ refers to the global analysis of epigenetic changes across the entire genome within a particular cell and cellular context [11, 14]. Aberrant patterns of gene expression are key features of cancer, and both genetic and epigenetic abnormalities are implicated in this deregulation. In the etiology of cancer it is now recognized that epigenetics and genetics can cooperate throughout the carcinogenesis process [12]. However, in contrast to genetic modifications, epigenetic deregulation is potentially reversible and intervention strategies targeting the epigenome have been proposed for both cancer prevention and therapeutics [9, 15, 16]. There are both in vitro and in vivo data showing that several BFCs can interfere with DNA methylation and histone modifications, and affect the expression of genes involved in the carcinogenic process. The ability of these dietary components to modulate the epigenetic machinery is viewed as an important feature of their anticancer actions [9, 10, 17]. Furthermore, early-life nutrition may have long-term consequences on cancer risk because diet-epigenome interactions can manifest during the in utero period [18, 19].

## Epigenetic Alterations in Cancer

While transitory control of gene transcription is mediated mainly by transcription factors, more permanent patterns of gene expression are established by epigenetic changes including methylation of cytosine bases in DNA and perhaps post-translational modifications of histones such as methylation. The dynamic interplay between these epigenetic alterations influences chromatin remodeling with either gene activation or silencing [20].

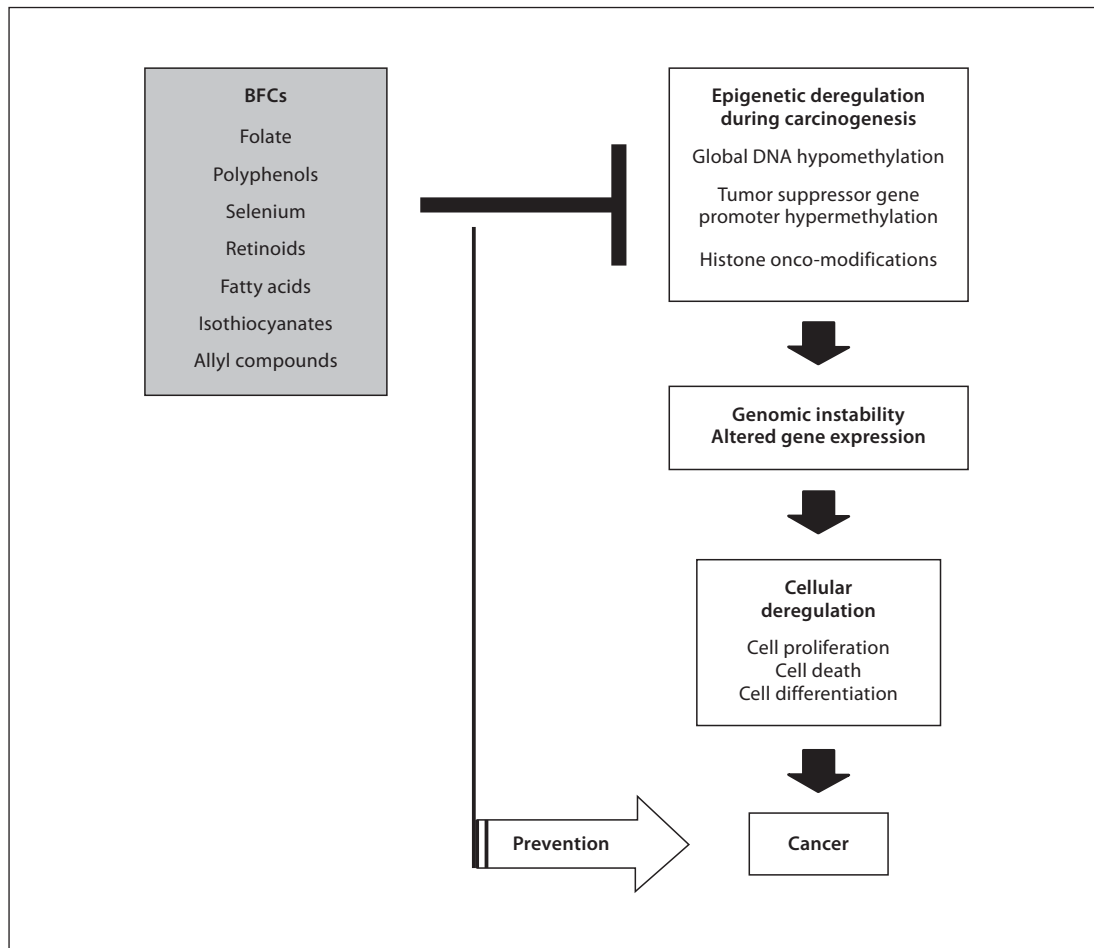
Global DNA hypomethylation is a frequent molecular alteration in human tumors [21]. In cancer, loss of DNA methylation occurs predominantly in the highly repeated DNA sequences that make up half of the human genome and include centromeric and pericentromeric tandem repeats, although it can also be found in gene promoters [21]. DNA hypomethylation is associated with the induction of chromosomal instability, reactivation and transposition of retrotransposable elements, loss of imprinting, and activation of normally silent genes, including oncogenes [22]. Epigenetic silencing of tumor suppressor genes due

to site-specific promoter hypermethylation is also a common feature of the cancer epigenome and represents an alternative to mutations as a cause of gene function loss. This epigenetic alteration can occur early in the neoplastic process and disrupt key cell signaling pathways favoring clonal expansion [23]. Genes frequently subjected to hypermethylation in cancer are associated with several processes, including DNA repair, carcinogen detoxification, cell cycle control, apoptosis, differentiation, angiogenesis and metastasis [24]. Examples include *p15<sup>INK4β</sup>*, *p16<sup>INK4a</sup>*, Ras association (RalGDS/AF-6) domain family member 1 (*RASSF1*), retinoic acid receptor β (*RARβ*), *O*<sup>6</sup>-methylguanine methyltransferase (*MGMT*) and glutathione S-transferase P1 (*GSTP1*) [25]. Reactivation of epigenetically silenced tumor suppressor genes with the demethylating agents 5-azacytidine and 5-aza-2'-deoxycytidine represents a promising anticancer strategy. These nucleoside analogues inhibit by covalent binding DNA methyltransferases (DNMTs), the enzymes that catalyze the transfer of a methyl group from the universal methyl donor S-adenosyl-L-methionine (AdoMet) to DNA [26].

Disruption of histone modifications has been implicated in carcinogenesis by their ability to induce aberrant gene expression, encourage loss of genomic instability, impair DNA repair and diminish cell cycle checkpoint stability; thus the terminology 'histone onco-modifications' was recently proposed to describe post-translational modifications linked to cancer [27]. Aberrant patterns of histone methylation are intimately implicated in tumor initiation and progression, and deregulation of H3K4me3 and H3K27me3 can be highlighted for such activity [28]. Furthermore, global loss of H4K16 acetylation and H4K20 trimethylation is a common hallmark of cancer [29]. The observation that these losses in H4 lysine acetylation or trimethylation occur in hypomethylated repetitive genomic regions [30], and that trimethylated H3K27 can be found in the context of hypermethylated tumor suppressor promoters [31], illustrates the dynamic and complementary deregulation of epigenetic processes during cancer development. Histone deacetylases (HDACs) have altered activities in diverse cancers and are considered promising epigenetic targets for anticancer interventions with HDAC inhibitors (HDACi) [15]. This is exemplified by vorinostat (suberoylanilide hydroxamic acid), which has been approved by the US Food and Drug Administration for cutaneous T-cell lymphoma treatment [32]. Global alterations in histone methyltransferases and histone demethylases leading to deregulation of both activating or repressing methylation marks are also thought to be involved in cancer development. Epi-drugs targeting methylation and demethylation pathways are now being considered for the reversal of epigenetic abnormalities in cancer cells [33].

### **Dietary Modulation of Epigenetic Processes**

Several BFCs with anticancer potential have been shown to influence epigenetic processes. These dietary molecules are constituents of several chemical classes and include folate, polyphenols, selenium, retinoids, fatty acids, isothiocyanates and allyl compounds [6–10, 15]. By interfering with epigenetic processes deregulated during carcinogenesis, such as global DNA hypomethylation, tumor suppressor gene promoter hypermethylation and histone onco-modifications, these BFCs could affect mechanisms relevant for cancer prevention, including signal transduction pathways, cell growth, differentiation and apoptosis, among others (fig. 1) [9, 17, 34].



**Fig. 1.** DNA methylation alterations and histone onco-modifications as promising targets for cancer prevention by BFCs.

### BFCs Affecting DNA Methylation

Interference at the DNA methylation level represents a promising mechanism of dietary modulation of gene expression for cancer prevention [7, 10]. BFCs can affect DNA methylation through at least four mechanisms. First, dietary factors are important sources of methyl groups necessary for AdoMet synthesis. Second, dietary factors can affect methyl group utilization by processes including shifts in DNMT activity. A third plausible mechanism could be related to DNA demethylation, although direct evidence is still lacking. Finally, DNA methylation patterns leading to activation or repression of genes related to cancer development could influence the response to dietary interventions [6].

#### *Methyl Donor Nutrients*

Labile methyl groups for DNA methylation reactions are supplied mainly by choline and methionine, and are regenerated endogenously by folate and vitamin B<sub>12</sub> in the 1-carbon metabolism pathway [35]. Dietary deficiency in these methyl donors and co-factors has been found to be carcinogenic by itself as development of hepatocellular carcinomas is observed in rats fed diets deficient in choline, methionine and folate [36]. Induction of both global DNA

hypomethylation and tumor suppressor gene promoter hypermethylation has been associated with methyl-deficient diet-mediated hepatocarcinogenesis in rats [37, 38]. A diet deficient in L-methionine and devoid of folic acid and choline altered the DNA methylation machinery by increasing the protein expression of DNMT1 and the methyl CpG binding proteins (MBD) 1, 2 and 4 at the early hepatic preneoplastic phase [39]. Animals fed a methyl-deficient diet have decreased levels of AdoMet [40]. Reconstitution of the methyl donor hepatic pool with AdoMet supplementation in these animals led to inhibition of preneoplastic lesions [41]. Folic acid administration (0.16 mg/100 g body weight) by gavage in diethylnitrosamine-induced hepatocarcinogenesis resulted in an increased AdoMet/S-adenosyl-L-homocysteine hepatic ratio and inhibition of *c-myc* expression but not a reversal of global DNA methylation in hepatic nodules [42]. Additionally, methionine (45 mg/100 g body weight i.p.) administration prevented the decreased methylation and increased expression of *c-myc* and *c-jun* oncogenes induced by trichloroethylene and its metabolites in the mouse liver [43]. Although folate deficiency has been related to different malignancies, the strongest evidence relates to colorectal cancer [44]. The effects of folate deficiency and supplementation on carcinogenesis and DNA methylation are rather complex and variable, and seem to be influenced by cell type, target organ, stage of transformation, and the degree and duration of folate depletion [45]. Animal studies have shown reduction in DNA methylation with low folate status and increased DNA methylation after folate supplementation for some tissues [46]. One intriguing feature of folate is its dual modulatory effect on colorectal carcinogenesis that depends on intervention timing and dosage. Modest folic acid (4–10 times above the basal daily requirement), but not supra-physiologic supplementation inhibits cancer development in normal colon mucosa, whereas it promotes the progression of established colorectal neoplasms; the main epigenetic effects involved in these paradoxical effects are not clear [45]. Along with timing and dosage, polymorphisms in genes associated with methyl group metabolism might also influence folate interactions with DNA methylation and colorectal cancer risk [47]. In a cohort-based study, folate was identified as a dietary factor associated with reduced promoter methylation in cells exfoliated from the airway epithelium of smokers [48]. Protection against promoter methylation of a panel of genes [*p16<sup>INK4a</sup>*, *MGMT*, death-associated protein kinase (*DAPK*), *RASSF1A*, paired box 5 $\alpha$  (*PAX5 $\alpha$* ), *PAX5 $\beta$* , GATA binding protein 4 (*GATA4*), and *GATA5*] associated with lung cancer risk was observed for folate ingestion (OR, 0.84 per 750  $\mu$ g/day; 95% CI, 0.72–0.99) [48]. On the other hand, higher red blood cell folate levels were associated with higher levels of estrogen receptor  $\alpha$  (*ER $\alpha$* ) and secreted frizzled-related protein-1 (*SFRP1*) CpG island methylation in normal colorectal mucosa of subjects enrolled in a multicenter chemoprevention trial testing the efficacy of folate on the risk of large bowel adenomas [49]. Because methylation in normal mucosa has been hypothesized to increase colorectal neoplasia risk, the issue of supplementary folate administration safety in healthy adults was highlighted [49].

### *Polyphenols*

Modulation of epigenetic processes has been identified as a relevant anticancer feature of dietary polyphenolic compounds [9]. (–)-Epigallocatechin-3-gallate (EGCG; 20  $\mu$ M), the main catechin (a polyphenol class) from green tea, has been shown to inhibit DNMT in human esophageal (KYSE 150), colon (HT29), prostate (PC3), and mammary (MCF-7 and MDA-MB-231) cancer cell lines [50]. These actions were associated with promoter demethylation and reactivation of *p16<sup>INK4a</sup>*, *RAR $\beta$* , *MGMT*, human mutL homolog 1 (*hMLH1*) and *GSTP1* [50]. In vitro studies showed that EGCG [0.47  $\mu$ M] was a more potent DNMT inhibitor than other bioactive green tea components [catechin (4.6  $\mu$ M) and epicatechin (8.4  $\mu$ M)] and bioflavonoids [quercetin (1.6  $\mu$ M), fisetin (3.5  $\mu$ M) and myricetin (1.2  $\mu$ M)] [51]. Computational modeling studies revealed that the gallic acid moiety of EGCG plays a crucial role in its high-affinity inhibitory interaction with the catalytic site of human DNMT1 [51]. In



MCF-7 human breast cancer cells, EGCG (100  $\mu\text{M}$ ) induced promoter demethylation and inhibited the expression of human telomerase reverse transcriptase (*hTERT*), the catalytic subunit of telomerase. This apparent paradoxical effect was associated with increased promoter binding of the *hTERT* transcriptional repressor E2F-1 due to site-specific demethylation [52]. Interestingly, lack of DNMT inhibition by EGCG (20–30  $\mu\text{M}$ ) has been reported in human bladder (T24), colorectal (HT29) and prostate cancer (PC3) cells [53]. Similarly, consumption of a green tea extract (0.3%) in drinking water did not induce DNA hypomethylation in prostate tissue of both healthy and transgenic adenocarcinoma of mouse prostate (TRAMP) evaluated by a genome-wide DNA methylation profiling assay [54]. In this study, lack of DNMT inhibition by the green tea extract could be due to low bioavailability and stability of polyphenols that would lead to insufficient retention of these BFCs in prostate tissue. In addition, these investigators also observed that the status of four loci hypermethylated during tumor progression was unaltered by green tea extract in TRAMP mouse prostates and that this treatment did not inhibit tumor progression in these animals. The findings in different laboratories regarding the efficacy of green tea polyphenols in the TRAMP model may be due to timing of exposure [54]. A few other *in vivo* studies have examined the ability of catechins to alter DNA methylation. Protective effects of green tea (0.6% in drinking water) in a mouse colon carcinogenesis model were associated with a decrease in retinoid X receptor  $\alpha$  (*RXR $\alpha$* ) promoter methylation [55]. Topical application of EGCG inhibited UVB-induced photocarcinogenesis and global hypomethylation in mouse skin tissue [56]. Treatment of patients with high-risk oral premalignant lesions with green tea extract (500–1,000 mg/m<sup>2</sup> thrice daily) did not reverse the hypermethylated status of the *p16<sup>INK4a</sup>* promoter in this tissue, although only 2 individuals were evaluated [57]. In an observational study, methylation of the homeobox transcription factor (*CDX2*) and bone morphogenetic protein 2 (*BMP-2*) genes in human gastric cancer tissue was inversely associated with past consumption of green tea (7 cups or more per day) [58].

Genistein, an isoflavone from soybeans, has also been shown to inhibit DNMT activity in different cancer cell lines [50]. The isoflavone (2–20  $\mu\text{M}$ ) reactivated *p16<sup>INK4a</sup>*, *RAR $\beta$*  and *MGMT* expression via demethylation of their promoters in KYSE 510 human esophageal squamous cell carcinoma cells [59]. Similarly genistein (20  $\mu\text{M}$ ) also reactivated *RAR $\beta$*  in LNCaP and PC3 human prostate cancer cells [59]. Genistein (50  $\mu\text{M}$ ) inhibition of A498, ACHN and HEK-293 human renal cancer cell growth was accompanied by promoter demethylation and reactivation of the tumor suppressor B-cell translocation gene 3 (*BTG3*) via inhibition of DNMT and MBD2 activities [60]. Few *in vivo* studies have investigated the modulatory effects of genistein on DNA methylation. In one study, genistein (300 mg/kg diet) altered the methylation profile of wild-type mouse prostate tissue [61]. In healthy premenopausal women, consumption of higher levels of genistein (90.6 mg/day) and daidzein (36.4 mg/day) for one menstrual cycle was associated with increased methylation of *RAR $\beta$*  and the cell cycle regulator cyclin D2 (*CCND2*) in breast samples, although the significance of these results is not clear [62].

Other dietary polyphenols and phenolic acids with anticancer potential have also been shown to affect DNA methylation. These include curcumin (turmeric), resveratrol (grapes), caffeic acid (coffee) and chlorogenic acid (coffee). DNA hypomethylation by curcumin (3 and 30  $\mu\text{M}$ ) in MV4–11 human leukemia cells was thought to involve the covalent blocking of the catalytic pocket of DNMT1 [63]. Resveratrol (10 and 20  $\mu\text{M}$ ) reversed epigenetic silencing of breast cancer 1 (*BRCA-1*) by the aromatic hydrocarbon receptor agonist 2,3,7,8-tetrachlorobenzo(p)dioxin in MCF-7 human breast cancer cells [64]. The *BRCA-1* promoter in resveratrol-treated cells showed reduced association of the aromatic hydrocarbon receptor, DNMT1 and MBD2 [64]. In MCF-7 and MDA-MB-231 breast cancer cells, caffeic acid (1–50  $\mu\text{M}$ ) and chlorogenic acid (1–50  $\mu\text{M}$ ) induced *RAR $\beta$*  promoter demethylation indirectly

through increased formation of S-adenosyl-L-homocysteine (a potent inhibitor of DNMT) during the catechol-*O*-methyltransferase-mediated *O*-methylation of these dietary catechols [65].

### Selenium

Evidence from animal and cell culture experiments shows that the anticancer effects of selenium may involve interference with DNA methylation [7, 66]. Deficiency in dietary selenium (0 mg/kg diet) caused global DNA hypomethylation in rat liver and colon tissues and in human colon cancer cells Caco-2 (0  $\mu$ M added selenium) in which methylation of the *p53* promoter region was also influenced by selenium status [67]. Selenium (selenite; 1 and 2  $\mu$ M) also inhibited expression of *DNMT1* in a human adenocarcinoma cell line (HT29), possibly by affecting the AP-1-regulatory region of *DNMT1* [68]. Selenium can also modulate DNA methylation by interfering with 1-carbon metabolism [69]. The chemical form of the essential element can further influence epigenetic modulation. Treatment of LNCaP prostate cancer cells with seleno-DL-methionine (150  $\mu$ M) did not alter the expression of *GSTP1* that was epigenetically silenced by promoter hypermethylation [70]. On the other hand, selenite (1.5  $\mu$ M) restored expression of *GSTP1* by inhibition of HDAC activity and downregulation of DNMT expression that was accompanied by reductions in global DNA methylation in LNCaP cells. Promoter analysis of this gene revealed decreased association of DNMT1 and methylation of H3K9, and increased acetylation of this histone residue in selenite-treated cells [66]. One recent study in humans showed that selenium plasma levels were inversely correlated with genomic DNA methylation in leukocytes [71]; the health implications of these findings require further study.

### Retinoids

Epigenetic modulation of gene expression has been suggested to be involved in the cancer prevention activity of retinoids [72]. Retinoids may alter the DNA methylation processes by enhancing 1-carbon metabolism. Treatment of rats by gavage with pharmacological doses (30  $\mu$ mol/kg body weight) of retinyl palmitate and its derivatives 13-*cis* and all-*trans* retinoic acids upregulated the hepatic activity of glycine N-methyltransferase, an enzyme essential for optimizing methyl group supply [73]. In this study, hepatic DNA from all-*trans* retinoic acid-treated rats was hypomethylated [73]. In another study, vitamin A (3  $\mu$ mol/kg body weight, by gavage)-induced inhibition of hepatocarcinogenesis progression did not involve reversal of *c-myc* oncogene and hydroxy-methylglutaryl coenzyme A (*HMGCoA reductase*) gene hypomethylation in rat liver tumors [74]. Cellular retinol-binding protein 1 (*CRBP1*) and *RAR $\beta$*  play a central role in retinoid signaling by functioning as an intracellular retinol transporter and as a nuclear receptor for retinoic acid, respectively. In several cancer cell lines and primary tumors, both genes were shown to be epigenetically silenced by promoter region hypermethylation [75]. Colorectal cancer patients in the highest quartile of dietary vitamin A intake (mean daily consumption 155  $\mu$ g; interquartile range 102–239  $\mu$ g) were more likely to present with unmethylated *CRBP1* and *RAR $\beta$*  [75]. Retinoic acid reversal of the transformed phenotype in NB4 acute promyelocytic leukemia cells involved the demethylation of *RAR $\beta$ 2* promoter with re-expression of this gene [76]. Inhibition of *DNMT* expression and activity was shown to mediate these epigenetic effects via the retinoid [77]. Similarly, in MCF-7 breast cancer cells, inhibition of *DNMT1* expression by retinoic acid (0.35  $\mu$ M) led to promoter demethylation and re-expression of *RAR $\beta$ 2* [78]. It has been shown that retinoic acid can further epigenetically modulate targets other than its nuclear receptor [79]. A comprehensive DNA methylation profiling approach of human embryonic stem cells treated with retinoic acid (50  $\mu$ M) revealed 166 differentially methylated CpG sites and 2,013 differentially expressed genes [79]. In another study also employing an epigenomic approach

in human embryonic stem cells, it was verified that vitamin C (50 µg/ml) treatment promoted genome-wide demethylation, but specifically affected a subset of 1,847 genes, including important stem cell genes [80].

### **BFCs Affecting Histone Acetylation and Methylation**

Diverse BFCs with cancer prevention potential regulate gene expression by altering post-translational modifications of histones [15]. These dietary constituents encompass butyrate, a short-chain fatty acid produced by bacterial fiber fermentation in the colon, present in honey and milk products, isothiocyanates from cruciferous vegetables, allyl sulfides from garlic, polyphenols from tea among other sources, selenium, retinoids and ω-3 fatty acids [8, 9, 34, 81–84]. Many of these constituents modulate HDAC activity, which is important because HDACi are being considered a promising class of agents for cancer treatment and may have utility for cancer prevention [15]. Other enzymes that modify histone residues and members of the co-repressor and co-activator complexes that associate with them represent important targets of the chromatin architecture [17].

#### *Butyrate*

Butyrate represents one of the first anticancer agents to be identified with histone acetylation properties, and its inhibitory effects on HDAC activity have been reported in several cell lines [15]. Butyrate is the smallest-identified HDACi, and it is made up of a simple 3-carbon ‘spacer’ attached to a carboxylic acid group. It is hypothesized that by entering the HDAC-active pocket, butyrate inhibits enzyme activity by forming a bidentate ligand of the carboxylate group with the buried zinc atom [8]. Among butyrate-responsive genes, *p21<sup>WAF1</sup>* and *Bax* have been highlighted [15]. For example, in Colo-320 human colon cancer cells treated with butyrate (5 mM), activation of *p21<sup>WAF1</sup>* expression was associated with increased H3 and H4 acetylation within its gene promoter region [85]. A model was proposed in which inhibition of Sp1-/Sp3-associated HDAC activity leads to induction of histone acetylation and transcriptional activation of *p21<sup>WAF1</sup>* [86]. It was verified that cell growth arrest at G<sub>2</sub> and apoptosis induction by butyrate (1–10 mM) in HCT116 human colon cancer cells were associated with *p21<sup>WAF1</sup>* and *Bak* overexpression [87]. More specifically, butyrate increased the acetylation of Sp1, and this resulted in decreased binding to these gene promoters, indicating that non-histone proteins also represent relevant targets for the anticancer actions of this HDACi [87]. Because of its HDAC-inhibitory effects, butyrate has been considered for combinatorial anticancer interventions with diverse agents, including dietary and synthetic DNMT inhibitors [88] and retinoids [89]. Few studies have investigated butyrate HDAC-inhibitory actions in in vivo models. A combination of sodium butyrate (1.5% in drinking water) and folic acid (8 mg/kg body weight) has demonstrated additive inhibitory actions in vivo on colon cancer development [90]. Induction of *p21<sup>WAF1</sup>* expression by sodium butyrate in colon tumor samples was associated with global H3 acetylation [90]. The short half-life of butyrate is, however, an important limitation to its clinical use. Tributyrin, a butyrate pro-drug made up of a glycerol molecule esterified with 3 butyrate molecules, has been proposed as an alternative based on its better pharmacokinetic profile. In a rat hepatocarcinogenesis model, tributyrin (200 mg/kg body weight) inhibited hepatic preneoplastic development when provided continuously during the initiation and promotion phases [91]. These chemopreventive activities involved the induction of hepatic H3K9 global acetylation and *p21<sup>WAF1</sup>* expression by HDACi [91]. Similar results were observed when tributyrin (200 mg/kg body weight) was provided alone or in combination with vitamin A (1 mg/kg body weight) specifically during the promotion phase of hepatocarcinogenesis [89].



### *Isothiocyanates*

Inhibition of HDAC activity with increased histone acetylation has been associated with sulforaphane and related dietary isothiocyanate employing both in vitro and in vivo models [8, 34]. Sulforaphane (15  $\mu\text{M}$ ) treatment inhibited HDAC activity and increased  $p21^{\text{WAF1}}$  and *Bax* expression that was accompanied by H4 acetylation within  $p21^{\text{WAF1}}$  and *Bax* promoters in colon (HCT116) [92] and prostate (LNCaP) [93] cancer cells. Importantly, this sulforaphane concentration is comparable to the isothiocyanate tissue concentrations (3–30  $\mu\text{M}$ ) that were associated with sulforaphane chemopreventive activities against spontaneous intestinal polyp development in the *Apc*<sup>min</sup> mouse [81, 94]. Normal and neoplastic cells show selective responses to treatment with isothiocyanate. Sulforaphane (15  $\mu\text{M}$ ) induced cell cycle arrest and apoptosis, decreased HDAC activity and class I and II HDAC proteins, increased acetylation of H3 within the  $p21^{\text{WAF1}}$  promoter and induced  $p21^{\text{WAF1}}$  expression in hyperplastic (BPH1) and cancerous prostate cells (LNCaP and PC3) but not in normal prostate cells (PrEC) [95]. In human breast cancer cells (MDA-MB-231, MDA-MB-468, MCF-7 and T47D) inhibition of HDAC and induction of apoptosis by sulforaphane (5–25  $\mu\text{M}$ ) was not accompanied by alterations in global H3 and H4 acetylation levels, although local histone modifications were not evaluated [96]. HDAC inhibition has been linked to the sulforaphane metabolite sulforaphane-cysteine that fits the enzyme pocket, with the  $\alpha$ -carboxyl group of the cysteine moiety forming a bidentate ligand with the buried zinc atom [81]. Chronic consumption of sulforaphane (443 mg/kg diet) by *Apc*<sup>min</sup> mice inhibited intestinal tumors and promoted increases in histone acetylation, both globally and within the promoter regions of  $p21^{\text{WAF1}}$  and *Bax* [97]. When provided in the diet to male nude mice, sulforaphane (average daily dose of 7.5  $\mu\text{mol}$ ) inhibited the growth of PC3 prostate cancer cells. The isothiocyanate-treated xenografts had decreased HDAC activity, increased *Bax* expression and a trend towards increased global acetylation of H3 and H4 [98]. Consumption by human subjects of a single dose of 68 g of broccoli sprouts, a sulforaphane-rich food, markedly inhibited HDAC activity in peripheral mononuclear blood cells after 3 and 6 h, with the enzyme activity returning to basal levels after 24 h [98]. Other isothiocyanates have also shown to affect HDAC activity and histone marks. Growth arrest of HL-60 leukemia cells by phenylhexyl isothiocyanate (5–40  $\mu\text{M}$ ) was associated with reduced HDAC activity, increased levels of the acetyl transferase p300 and increased acetylation of  $p21^{\text{WAF1}}$  [99]. LNCaP prostate cancer cells treated with phenethyl isothiocyanate (0.5–10  $\mu\text{M}$ ), present in watercress, showed cell growth arrest and HDAC activity inhibition [100]. Moreover,  $p21^{\text{WAF1}}$  upregulation by the isothiocyanate involved hyperacetylation and reduced binding of c-Myc to Sp1 transcriptional complexes in this gene promoter [100].

### *Allyl Compounds*

Diallyl disulfide, an organosulfur compound present in garlic and other *Allium* vegetables, has shown HDAC-inhibitory actions in different cellular and in vivo systems. Previous studies showed inhibition of HDAC activity with increased global H3 and H4 acetylation in DS19 and K562 leukemic cells treated with diallyl sulfide [101]. Increased histone acetylation was also described in liver and Morris hepatoma 7777 after treatment of rats with this organosulfur compound (200 mg/kg body weight) [102]. Growth arrest at G<sub>2</sub>/M after treatment of Caco-2 colon cancer cells with diallyl sulfide (200  $\mu\text{M}$ ) was associated with increased  $p21^{\text{WAF1}}$  expression [103]. This organosulfur compound inhibited HDAC activity and increased H3 acetylation, predominantly of lysine residue 14 [103]. Further analysis showed that treatment of Caco-2 and HT29 colon cancer cells with diallyl sulfide (200  $\mu\text{M}$ ) also increased acetylation of H3 and/or H4 within the  $p21^{\text{WAF1}}$  promoter region [104]. Diallyl disulfide inhibition of leukemia cell growth in vitro (1.25  $\mu\text{g/ml}$ ) and in vivo (42 mg/kg body weight) involved induction of  $p21^{\text{WAF1}}$  expression and induction of H3 and H4 acetylation

[105]. Diverse organosulfur compounds from garlic, including alliin, allicin, S-allylcysteine, S-allyl mercaptocysteine, diallyl sulfide, diallyl disulfide and diallyl trisulfide, can be metabolized to allyl methyl sulfide, methyl mercaptan and allyl mercaptan. This latter metabolite has been identified as the most active HDACi, and it was proposed that metabolic conversion of allyl sulfides to HDACi in situ would contribute to cancer prevention effects of garlic [81, 106]. HT29 colon cancer cells treatment with allyl mercaptan (0.5–2 mM) resulted in growth arrest at G<sub>1</sub> and in HDAC activity inhibition that was accompanied by increased global histone acetylation. Induction of *p21<sup>WAF1</sup>* expression by this organosulfur compound was associated with hyperacetylation and enhanced binding of Sp3 and p53 to the *p21<sup>WAF1</sup>* promoter region [106]. Because allyl compounds have been shown to modulate HDAC activity at relatively high concentrations (0.2–2 mM), it remains to be elucidated whether intracellular concentrations of allyl mercaptan on the order of 20 μM, which have been associated with HDAC inhibition in colonic epithelial cells and in prostate tissue, could be achieved after ingestion of multiple organosulfur compounds in garlic [106].

### *Polyphenols*

Cancer prevention by dietary polyphenols has been suggested to involve modulation of histone modifications [9, 17]. In LNCap and PC3 prostate cancer cells, genistein (50 μM) reactivated the expression of silenced tumor suppressor genes that presented unmethylated promoters, including phosphatase and tensin homolog (*PTEN*), cylindromatosis (turban tumor syndrome; *CYLD*), *p53* and fork head transcription factor 3a (*FOXO3a*) [107]. These actions occurred via demethylation and acetylation of H3K9 at the *PTEN* and *CYLD* promoters. In addition, genistein inhibition of the HDAC SIRT1 activity led to *p53* and *FOXO3a* promoter acetylation [107]. Genistein (10–25 μM) also induced *p21<sup>WAF1</sup>* and *p16<sup>INK4a</sup>* by increasing acetylation of H3, H4 and H3K4 at the transcription start sites of these genes and increased HAT expression in LNCaP and DuPro prostate cancer cells [108]. Inhibition of *hTERT* transcription by genistein (50–100 μM) in breast MCF10AT benign cells and MCF-7 cancer cells involved increased H3K9 trimethylation and decreased H3K4 dimethylation [109]. Curcumin has also been shown to affect histone post-translational modifications by inducing histone hypoacetylation in glioma cells [110].

### *Selenium*

Metabolism of the major dietary forms of selenium methylselenocysteine and selenomethionine can generate β-methylselenopyruvate and α-keto-γ-methylselenobutyrate, respectively, that comprise seleno-α-keto acids that have been identified as novel HDACi [111]. These organoselenium metabolites induced H3 hyperacetylation (10 and 50 μM for both compounds) in different prostate cancer cells (LNCaP, LNCaPC4-2 and PC3) and inhibited HDAC activity in nuclear fractions of these cells (0.025–2.5 mM for β-methylselenopyruvate and 0.25–2.5 mM for α-keto-γ-methylselenobutyrate) [112]. These data indicate that selenium metabolism in cancer cells can influence histone modifications and should be considered in intervention strategies.

## **BFCs Affecting Polycomb Group Proteins**

The polycomb group (PcG) proteins, containing at least two distinct complexes, PcG complex 1 and 2, function as transcriptional repressors to silence specific sets of genes through coordinated chromatin modification [113]. PcG complex 2, composed of several factors/proteins including histone methyltransferases, is first recruited to silence chromatin with concomitant methylation of histone H3K27me3. This is followed by PcG complex 1 re-

cruitment through recognition of the H3K27me3 mark, which thereby triggers ubiquitination of histone H2A and/or inhibits chromatin remodeling to maintain the silenced state of the locus. Knockout mice lacking components of the PcG complex have suggested that these chromatin-repressive complexes are essential for sustaining stem cell activity [114]. Deregulation of the component proteins have been shown to contribute to the pathogenesis of cancer [115]. Investigators are beginning to examine the interplay between BFCs, PcG complexes and their impact on cancer prevention.

#### *Retinoids*

Retinoic acid primary target genes, such as homeobox A1 (*Hoxa1*), *Cyp26a1*, and *RARβ2*, are coated with PcG proteins in untreated stem cells. Following retinoic acid addition to stem cells, there is a rapid dissociation of the PcG proteins from these retinoic acid target genes by a mechanism that is not fully understood [116]. Investigators have recently delineated the differences in retinoic acid-mediated transcriptional responses and the underlying epigenetic status of these same genes (*Hoxa1*, *Cyp26a1* and *RARβ2*) in embryonal carcinoma cells and fibroblasts [83]. In the embryonal carcinoma cells, retinoic acid (1 μM) reduced the PcG 2 protein Suz12 and the associated H3K27me3-repressive epigenetic modification at the RAREs of *Hoxa1*, *Cyp26a1* and *RARβ2*. In contrast, in Balb/c3T3 fibroblast cells cultured with/without retinoic acid, Suz12 was not associated with the *Hoxa1*, *RARβ2*, and *Cyp26a1* RAREs, and only low levels of the H3K27me3 mark are seen at these RAREs. Therefore, Suz12 was not required for gene repression in the absence of retinoic acid in these cells. These investigators are beginning to unravel the complex mechanisms, including epigenetic processes, which control retinoic acid-mediated transcription in stem cells compared to other cell types.

#### *Polyphenols*

The protein B-cell-specific Moloney murine leukemia virus integration site 1 (BMI1), a component of the PcG complex 1, is markedly elevated in some human cancers, including epidermal squamous cell carcinoma [117]. The polyphenol EGCG (40 μM) was found to suppress BMI1 levels and reduce BMI1 phosphorylation, resulting in displacement of the BMI1 polycomb protein complex from chromatin and decreasing survival of skin cancer cells [118]. More recently, these investigators examined the effect of EGCG on BMI1 and the PcG complex 2 component enhancer of zeste homolog 2 (EZH2), which is also overexpressed in cancer cells [119]. EGCG treatment (60 μM) of SCC-13 skin cancer cells reduced both BMI1 and EZH2 levels, which was associated with reduced cell survival and global reduction in histone H3K27me3, a hallmark of PcG complex 2 action. The changes in BMI1 and EZH2 were also associated with reduced expression of key proteins that enhance progression through the cell cycle, increased expression of proteins that inhibit cell cycle progression, and impacted expression of proteins involved in apoptosis. This study is important because it suggests that green tea polyphenols reduce skin tumor cell survival by influencing PcG-mediated epigenetic regulatory mechanisms.

Several studies have pointed to the modulation of various molecular targets by curcumin. Recently, investigators observed a dose- (from 0 to 75 μM for 6 h) and time- (50 μM for 0 to 6 h) dependent downregulation of expression of the PcG protein EZH2 by curcumin in the MDA-MB-435 metastatic cancer cell line [120]. Curcumin was also found to inhibit EZH2-mediated H3K27 methylation in these cells, suggesting a new mechanism for the anticancer activity of curcumin.

#### *Fatty Acids*

Dietary ω-3 fatty acids have been shown to influence the development and progression of breast cancer and recent evidence shows their involvement in regulating the expression of

the PcG protein EZH2 in different breast cancer cell lines [84]. Treatment of several breast cancer cells (MDA-MB-231, T47D and MCF7 cells) with  $\omega$ -3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid at doses of 40 and 80  $\mu$ M) led to downregulation of EZH2, a decrease in H3K27 trimethylation, and a decrease in the invasive phenotype of the cells. Additional studies suggested that this downregulation of EZH2 was due to degradation of the PcG protein through post-translational mechanisms. Interestingly,  $\omega$ -6 fatty acids (linoleic acid and arachidonic acid) had no effect on the expression of EZH2. These findings suggest that the PcG protein EZH2 is an important target of  $\omega$ -3 fatty acids and that downregulation of EZH2 may be involved in the mediation of anticancer effects of  $\omega$ -3 fatty acids. Future research is needed to clarify the role of dietary regulation of PcG complexes during the carcinogenic process. The importance of the polycomb-repressive complexes in the development of cancer is currently an active research topic with much of the work focused on PcG complex involvement in acquiring and maintaining cancer stem and adult stem cell identification. Evidence also suggests that the PcG-targeted genes in normal cells may be more likely to acquire aberrant promoter hypermethylation in cancers [121]. Investigators will likely gain an understanding of how the gene silencing process goes awry by dissecting the factors that control PcG targeting. How dietary factors participate in this process is an emerging area of research.

### **Fetal Origins of Cancer: Importance of Epigenetic Programming by Nutritional Interventions during Early Life**

Cancer may have a developmental origin, and perturbations in the fetal environment have been hypothesized to program disease risk in later life [19, 122]. The inability of the fetal liver to metabolize and detoxify toxicants efficiently, the immaturity of fetal mechanisms of DNA repair and immune response, and the high rate of cell proliferation are important factors modulating the susceptibility of the fetus to environmental exposures including carcinogens and nutritional factors [123]. For example, increased risk of vaginal cancer among the daughters of mothers who had consumed diethylstilbestrol during pregnancy for prevention of miscarriage has been reported [124]. Breast cancer has also been related to fetal exposure to hormones and nutrients [18, 125]. The hypothesis that breast cancer is initiated in utero as a result of increased fetal exposure to maternal estrogen levels was proposed by Trichopoulos [126] based on population studies showing an association with high birth weight and increased risk for breast cancer in adult life. Studies conducted in rat models show that nutritional interventions with high-fat diets, genistein or alcohol during pregnancy can increase the susceptibility of female offspring to carcinogen-induced mammary carcinogenesis by altering mammary gland differentiation and expression of genes related to cell proliferation and apoptosis [18, 127]. Embryogenesis is characterized by extensive epigenetic reprogramming, and improper modulation of the epigenome during this highly sensitive period may have short- and long-term effects on the newborn and his/her progeny [128]. Thus, epigenetic marks could represent the mechanism whereby an adult organ's genome would retain the memory of early-life environmental exposures, including nutrition, by long-term alterations in gene expression programming [129]. Dietary and hormonal modulation of the fetal epigenome has been implicated in later risk for breast cancer [18, 130]. Results from one study showed promoter hypomethylation of *ER- $\alpha$*  in the mammary tissue of young female rats exposed in utero to a high- $\omega$ -6 polyunsaturated fatty acid diet shown to increase breast cancer risk compared to non-exposed female rats [131]. Although few studies have addressed the impact of nutritional modulation of the epigenome specifically in the context of in utero programming of breast cancer, there is in-



creasing evidence in other models that BFCs interfere with DNA methylation and histone modifications during early life. The viable yellow agouti ( $A^{vy}$ ) mouse model has been particularly useful in this regard. Maternal dietary supplementation with methyl-group donors, including folic acid, vitamin B<sub>12</sub>, choline and betaine [132], or with genistein [133] shifted the coat color distribution of  $A^{vy}/a$  offspring toward pseudoagouti (brown). Increased CpG methylation at the  $A^{vy}$  locus has been thought to mediate these phenotype alterations. Using another model system, adult offspring of rats fed a protein-restricted diet during pregnancy show reduced promoter methylation and increased expression of the glucocorticoid receptor (GR) and peroxisomal proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) genes, which was prevented by folic acid supplementation [134]. Hypomethylation of the GR promoter was associated with reduced expression of DNMT1 but not DNMT3A/B [134]. A more detailed analysis of the GR promoter revealed reduced binding of MeCP2, and histone marks associated with transcription activation were increased (acetylation of H3K9 and H4K9 and methylation of H3K4), whereas those associated with transcription repression were decreased (dimethylation and trimethylation of H3K9) [135]. Excessive maternal nutrient intake may also impact the offspring's epigenome. This concept was tested in primates [136]. Maternal consumption of a high-fat diet leading to obesity was associated with increased H3K14 acetylation status in the fetal liver of primates. Dietary reversal of epigenetic alterations set by early environmental exposure represents a potential strategy for cancer prevention [127]. Moreover, because additional windows of susceptibility to environmental modulation of the epigenome include childhood and puberty [137], inadequate dietary patterns during this specific period of development could also influence cancer risk later in life.

## Conclusions

Evidence mainly from in vitro studies indicates that the cancer prevention activity of BFCs involves modulation of epigenetic processes. Dietary components including polyphenols, isothiocyanates, allyl compounds, folate, selenium, retinoids and fatty acids including butyrate and  $\omega$ -3 affect global DNA hypomethylation, tumor suppressor gene promoter hypermethylation and histone onco-modifications in different cancer cells. By interfering with epigenetic processes deregulated during tumor development, BFCs could influence transcriptional programs and affect DNA repair, oxidative stress, inflammation, cell growth, differentiation and apoptosis, among other processes. The reversible nature of epigenetic processes and the observation that aberrations in DNA methylation and histone modifications are early events in carcinogenesis emphasize the relevance of the epigenome as a promising target of BFCs for cancer prevention strategies. However, studies specifically addressing their effects in animal models of human disease as well as in humans are warranted. Important factors that should be elucidated include the necessary dose and timing of BFC intervention to attain cancer-preventive epigenetic effects. The duration and specificity of epigenetic modulation by these dietary agents are also important topics in nutritional epigenomics and cancer research. Because diet-epigenome interactions are likely to occur in utero, the impact of early-life nutrition on cancer risk programming should be investigated further. Other susceptible windows during the lifespan (i.e. childhood and puberty) warrant investigation as well. Finally, as most of the evidence for dietary influence on DNA methylation and histone modifications is based on epigenetic effects on candidate genes, there is a need for additional studies investigating the response of the epigenome to BFC treatment on a genome-wide scale and the consequences for cancer prevention.



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