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Epigenetics: A New Link Between Nutrition and Cancer

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REVIEW ARTICLE

Epigenetics: A New Link Between Nutrition and Cancer

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Emerging studies suggest that dietary components can affect gene expression through epigenetic mechanisms. Epigenetic modifications are heritable and potentially reversible changes in gene expression that do not require changes in the DNA sequence. The main mechanisms of epigenetic control in mammals are DNA methylation, histone modifications, and RNA silencing. The potential reversibility of epigenetic changes suggests that they could be modulated by nutrition and bioactive food compounds. Thus, epigenetic modifications could mediate environmental signals and provide a link between susceptibility genes and environmental factors in the etiology of cancer. Elucidating the impact of nutrition on epigenetic mechanisms may serve as a tool to predict an individuals' susceptibility to cancer, provide dietary recommendations, or provide therapeutic applications of natural compounds against cancer. The optimal duration and the dose necessary for a chemopreventive effect require further studies. There is limited information about tissue specificity and temporal aspects of dietary treatments. Species differences need to be considered when interpreting results from various models. Importantly, molecular mechanisms of bioactive dietary components should be investigated in greater detail in human intervention studies. Although some of these issues remain controversial, this review mainly focuses on promising data that support the developing field of Nutritional Epigenetics.

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INTRODUCTION

Recent studies provide evidence that dietary components may affect the process of carcinogenesis. Isothiocyanates from cruciferous vegetables (cauliflower, cabbage, and broccoli), diallyl sulfide (an organosulphur compound from garlic), isoflavone, phytosterole, folate, selenium, vitamin E, flavonoids and dietary fibers, may reduce the risk of cancer. Emerging evidence suggests that the protective effects can be mediated through epigenetic mechanisms. Epigenetic modifications are heritable changes in gene expression that do not require changes in the DNA sequence (1). The main mechanisms of epigenetic control in mammals are DNA methylation, histone modifications, and RNA interference (RNA silencing) (2). The present review gives a comprehensive overview of the current literature on bioactive components and their influence on the major epigenetic mechanisms in cancer, with a focus on compounds that influence multiple epigenetic mechanisms.

Overview of DNA Methylation Changes in Cancer

The key epigenetic modification in mammals is the addition of a methylgroup to the carbon-5 position of cytosine in a CpG dinucleotide sequence (1,3). CpG dinucleotides are often clustered in CpG-rich regions known as *CpG islands* that are frequently associated with the transcription start sites (2,3). Hypermethylation of these regions can lead to transcriptional silencing of tumor suppressor genes, causing their inactivation and malignant transformation in several cancer types (1). The covalent addition of a methyl group is catalyzed by the DNA methyltransferases (DNMTs) family of enzymes that use S-adenosyl-methionine (SAM) as the universal methyl donor (3), Fig. 1. While DNMT1 is primarily involved in the maintenance

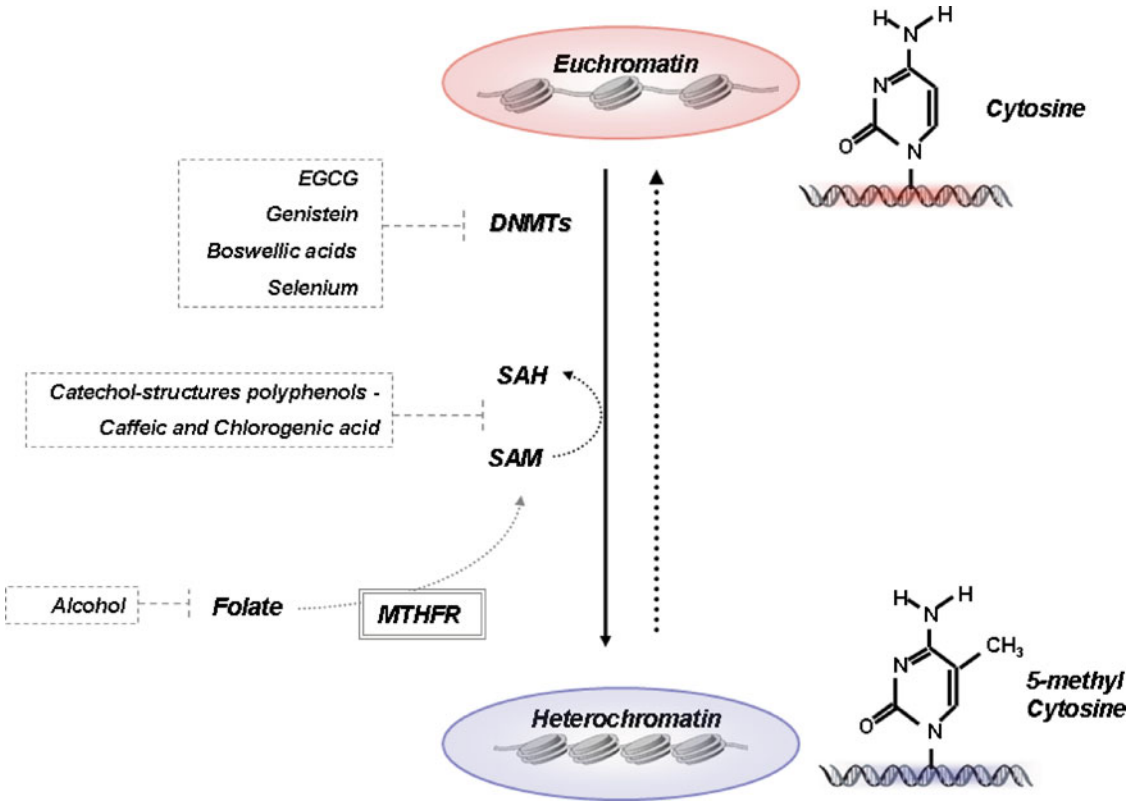

FIG. 1. DNA methylation of cytosine located 5′ to guanosine of CpG islands in promoters lead to transcriptional silencing of tumor suppressor genes and malignant transformation. This reaction is catalyzed by the DNA methyltransferases (*DNMTs*), with *S*-adenosyl-methionine (*SAM*) as a universal methyl donor. The dietary polyphenols, epigallocatechin-3-gallate (EGCG) from green tea, genistein from soybean and isothiocyanates from plant foods, are bioactive food components with cancer prevention properties. Cancer inhibition generated from dietary polyphenols is associated with gene reactivation through demethylation in the promoters of methylation-silenced tumor suppressor genes. The effects of dietary polyphenols such as EGCG on *DNMTs* appear to have their direct inhibition by interaction with the catalytic site of the *DNMT1* molecule, while catechol-structures polyphenols - caffeic acid and chlorogenic acid are affecting the bioavailability of SAM. (Color figure available online).

of DNA methylation after replication, DNMT3A and DNMT3B interact with the transcription machinery and mediate *de novo* methylation. Several studies have shown overexpression of DNMTs (mainly DNMT1 and DNMT3B) in multiple cancers (3).

Dietary Components, DNA Methylation, and Transgenerational Epigenetic Changes

A number of epidemiological studies associate adverse environmental conditions and nutrition early in development, during prenatal development or adolescence with risk for developing a disease in adulthood. Although the mechanisms underlying this association are not fully elucidated, emerging evidence suggests involvement of epigenetic dysregulation (4–7). Exposure to severe conditions during prenatal development and adolescence can result in epigenetic changes that persist later in life.

Particularly intriguing are studies performed on individuals and their offspring who experienced the Dutch Hunger Winter of 1944–1945. Individuals exposed to severe famine during childhood and adolescence had a decreased risk of developing colorectal cancer (5), concurring with changes in the methylation of a panel of cancer-related genes (the CpG island methylator phenotype, or CIMP) (8). Exposure to energy restriction during critical periods of growth and development could result in a lower cancer risk through epigenetic changes (5). In addition, individuals exposed to the Dutch Hunger Winter during prenatal development had lower DNA methylation in the imprinted insulin-like growth factor 2 (*IGF2*) gene 60 yr later compared to their unexposed same-sex siblings (6). The influence of environmental factors and nutrients on epigenetic changes has also been studied in experimental models under controlled conditions (9). In Avy/a mice, maternal diet supplemented with methyl donors resulted in hypermethylation of a transposon in the promoter of the agouti gene that altered coat color in offspring mice (10,11). This effect persisted in the F2 generation suggesting possible germline changes. Nevertheless, real transgenerational inheritance of epigenetic changes induced during embryonal development requires that the changes persist in at least the F3 generation. One of the best examples to date is an increase in several pathologies, including breast cancer, that persisted for 4 generations in rats exposed to the endocrine disruptor vinclozolin (12). These studies indicate that the environmental conditions during early development are crucial for

establishing and maintaining epigenetic changes that can persist throughout life and can modulate the risk for complex diseases.

Dietary Components and DNA Methylation in Cancer

A growing body of evidence suggests that dietary plant-derived compounds, including folate, tea polyphenols, soy isoflavones, and polyphenols with catechol structures, have anticarcinogenic properties that can be mediated through DNA methylation (13,14) (see Fig. 1).

Folate, a water-soluble B vitamin that is present in green leafy vegetables, is involved in 1-carbon metabolism, DNA synthesis, and DNA methylation. Extensive evidence suggests that folate deficiency plays a significant role in the development of multiple cancers (15). Folate deficiency can mediate carcinogenesis through DNA damage (uracil misincorporation) (16,17), aberrant global or promoter methylation (18,19), and DNMT1 inhibition (20). Supplementing the diet with folic acid or natural folates marginally reduces the risk of colorectal cancer (21,22). Conversely, folate can promote cancer progression. High doses of folate supplementation (20 mg folate/kg) is associated with a substantial reduction in the number of ileal polyps compared with a low folate supplementation in *Apc* +/− mice after 3 mo of dietary intervention. However, folate supplementation had the opposite effect on the number of ileal polyps after 6 months of supplementation (23). Daily supplementation of human subjects with a history of colorectal adenoma with 5 mg folic acid and 1.25 mg vitamin B-12 increased uracil misincorporation into DNA and is associated with a tendency towards promoter methylation (16). One study reported that supplementation with 5 mg of folate during pregnancy is associated with an increased maternal mortality from cancer (24), although these findings have been questioned.

In addition, folate inadequacy does not influence all tissues equally. A recent study demonstrated that maternal low-folate status is associated with aberrant DNA methylation in human neural tube defects in a tissue-specific manner. Low maternal serum folate is associated with DNA hypomethylation in the brain and DNA hypermethylation in the skin and heart of fetuses with neural tube defects compared to controls (25). In a methyl deficient model of multistage hepatocarcinogenesis in rats, DNA hypomethylation and endogenous activity of DNMT progressively increased with time only in the tissues that undergoes carcinogenesis (26). Mice deficient in methylenetetrahydrofolate reductase (MTHFR) exhibit tissue-specific distribution of folates and a higher percentage of 5-methyl tetrahydrofolate in the brain compared to the liver (27), which could result in tissue-specific potential for DNA methylation in vivo.

The global content of 5-methyl cytosine and DNA methylation status are related to nutritive availability of methyl-donor compounds. High concentration of folate (20 μmol/l) enhanced cancer cell growth in *Caco-2* cells and concomitantly increased methylation of the estrogen receptor 1 (*ESR1*), *p16*, and *p15* promoters (28). Colorectal cancer patients with *p16* methylation consume significantly less folate, vitamin A, vitamin B1, potassium, and iron than controls, whereas patients with *p14* or *hMLH1* methylation consume significantly less vitamin A (29). Hypermethylation of estrogen receptor (ER)-alpha is associated with plasma levels of total homocysteine and shows inverse correlation with plasma levels of folate and vitamin B12 in primary breast cancer (30). This suggests that different methyl-donors could have different effects in enhancing carcinogenesis by inducing genetic or epigenetic changes.

Several studies have established a connection between cancer susceptibility and methyl-group metabolism genes (31), including *MTHFR*, a key enzyme involved in folate metabolism and DNA synthesis. *MTHFR* C677T genotype is associated with *RASSF1A* promoter hypermethylation in bladder and oral cancers (32,33). Carriers of the 677T allele of the *MTHFR* gene among patients with colorectal, breast, or lung tumors show constitutive low levels of 5-methylcytosine and global hypomethylation in tumors. The same study revealed that tumors from patients with methionine synthase 2756GG genotype showed promoter hypermethylation in a large panel of tumor suppressor genes, including *p16*, *p14*, *hMLH1*, *MGMT*, *APC*, *DAPK*, *GSTP1*, *BRCA1*, *RAR-β 2*, *CDH1*, and *RASSF1* (34). These findings suggest that single nucleotide polymorphisms in genes encoding enzymes involved in folate metabolism can modulate the epigenetic effects of environmental factors.

Interaction between polymorphisms in 1-carbon metabolism-related genes and environmental factors might lead to aberrant DNA methylation. This has been the most extensively demonstrated for alcohol in the Netherlands Cohort study (19). Higher frequency of promoter methylation of specific genes involved in colorectal carcinogenesis (*APC*, *p14*, *p16*, *hMLH1*, *O^6-MGMT*, and *RASSF1A*) was observed in patients with low folate (<215 μg/day) and high alcohol intake (≥5 g/day) compared to patients with high folate (≥215 μg/day) and low alcohol intake (0–4 g/day) (19). An interaction between heavy drinking and the *MTHFR* 667TT genotype in oral cancer causes multiple DNA methylation of tumor suppressor genes (33). However, the potential role of 1-carbon metabolism-related polymorphisms, interaction with dietary intake of methyl-donors and alcohol in regulating DNA methylation in cancer, has not yet been fully elucidated. Possible mechanisms by which alcohol could induce aberrant DNA methylation include ethanol-associated folate deficiency, interaction with 1-carbon metabolism, impairment of methyl group synthesis, and the synthesis of the universal methyl donor SAM (35). Further investigations are needed to provide recommendations for methyl-donors intake depending on the individuals' genetic susceptibility and exposure to known risk factors.

Epigallocatechin-3-gallate (EGCG), a major polyphenol ingredient of green tea with antioxidant activity, has been shown to inhibit tumor invasion and angiogenesis. Several in vitro, in vivo, and epidemiological studies have reported that the consumption of green tea may decrease cancer risk (36). In addition, long-term consumption of green tea (>30 yr) and drinking large quantities of green tea (≥250 g/mo) may decrease hepatocellular cancer risk (37). However, other studies showed limited data supporting these findings (36). In recent years, an

association was observed between green tea consumption and a lower incidence of gastric, esophageal, breast, ovarian, pancreatic, skin, and colorectal cancer (14). EGCG can inhibit DNMT through direct and indirect mechanisms (38). Treatment of human esophageal cancer *KYSE 510* cells, human colon cancer *HT-29* cells, and prostate cancer *PC3* cells with 20 and 50 μM of EGCG for 48 h caused a concentration- and time-dependent reversal of hypermethylation of p16, RARβ, MGMT, and hMLH1 genes (39). Treatment of *Caco-2* cells with 100 μmol/l EGCG induced cell growth inhibition and suppressed promoter methylation of tumor suppressor genes *p16* and *p15* (28). EGCG treatment of *MCF-7* breast cancer cells and *HL60* promyelocytic leukemia cells in a dose of 100 μM and 50 μM, respectively, reduced cellular proliferation and induced apoptosis in both cell lines in vitro (40). However, human telomerase reverse transcriptase (hTERT) mRNA expression was decreased only in *MCF-7* cells through decreased methylation of its promoter (40). These data indicate that EGCG may be effective in different cancer cell types through different pathways involving both anti-oxidant effects and epigenetic modulation.

In oral carcinoma cells, treatment with 50μM of EGCG for 6 days decreased methylation of the *RECK* gene and cancer cell invasion (41). In *H460* and *A549* lung cancer cell lines, treatment with 20 μM of EGCG for 72 h induced growth inhibition of lung cancer cells, *Wnt* inhibitory factor-1 (*WIF-1*) promoter demethylation and restoration of *WIF-1* expression (42). Treatment of human melanoma cells *A431* with 20 μM of EGCG for 6 days decreased global DNA methylation levels in a dose-dependent manner. In addition, EGCG decreased the levels of mRNA, protein, and activity of DNMT1, DNMT3A, and DNMT3B and induced reexpression of the mRNA and proteins of silenced tumor suppressor genes, *p16* and *p21* (43). There is no evidence of adverse effects of regular consumption of green tea. However, the potential harmful effects of EGCG overconsumption raise concerns. High amounts of this polyphenol could theoretically trigger DNA hypomethylation and reactivation of oncogenes and induce genomic instability.

Genistein, a soy isoflavone, has been shown to have chemopreventive properties through epigenetic mechanisms (44). Genistein reversed aberrant DNA methylation in doses of 2–20 μmol/L and reactivated *RARβ*, *p16*, and *MGMT* genes in *KYSE 510* cells (56). In higher doses of 20–50 μmol/L, genistein displayed a dose-dependent inhibition of DNA methyltransferase activity in esophageal cancer *KYSE 150* cells and prostate cancer *LNCaP* and *PC3* cells (45). Genistein treatment of *Caco-2* cells in a dose of 200 μmol/L increased promoter methylation of *ESR1* (28). In prostate cancer cell lines, genistein treatment induced demethylation of glutathione S-transferase P1 (*GSTP1)* and ephrin B2 (*EPHB2*) tumor suppressor promoters, which was followed by an increase in their protein expression (46). These findings indicate that genistein reactivate methylation-silenced tumor suppressor genes, partially through a direct inhibition of DNA methyltransferase, which may contribute to the development of dietary strategies and therapy based on natural isoflavones. However, effective doses and dose timing should be elucidated.

Resveratrol, a natural phytoalexin compound found in grapes, mulberries, and red wine, has cell growth-inhibitory activities. Increased *p16* methylation, but decreased *p15* methylation, was observed in *Caco-2* cells after exposure to 10 μmol/l resveratrol (28). After 48 h exposure to 30 μM resveratrol, an increase in *BRCA1* and *BRCA2* mRNA was observed in breast cancer cell lines *MCF7*, *MDA-MB 231*, and *HBL 100*, without changes at the protein level of these genes (47). Recently, it has been shown that resveratrol (at concentrations of 10 and 20 μmol/L) prevents the recruitment of DNMT1 to the *BRCA-1* promoter and induces silencing in *MCF-7* breast cancer cells (48).

Curcumin is a flavonoid from the rhizome of the plant *Curcuma longa* with anticancer activity. A recent study showed that treatment with 20 μM curcumin and genistein caused reversal of *RARβ2* gene hypermethylation in cervical cancer cell lines *SiHa* and *HeLa*, with a progressive demethylation as the time period of treatment was increased from 72 h to 6 days (66).

Quercetin, a dietary flavonoid with antioxidant and antiproliferative activities, is a natural inhibitor of catechol-O-methyltransferase. Quercetin induces cell cycle arrest and apoptosis in hamster buccal pouch tumors that correlates with the inhibition of DNMT1 (50). Quercetin also increases bioavailability of green tea polyphenols in vitro in *A549* and *786-O* cells, and in vivo, in immunodeficient (SCID) mice treated with 0.4% quercetin for 2 wk (51). In addition, quercetin increased antiproliferative activity of EGCG by increasing the intracellular concentration of EGCG and decreasing EGCG methylation in prostate cancer cells (52).

Butyrate is a fatty acid generated in the colon by the fermentation of dietary fibers, with potential cancer prevention activities (53). Butyrate induced promoter demethylation and reactivation of *RARβ2* following 24 h treatment in colon cancer *HT-29* and *HCT 116* cells (54). Interestingly, butyrate does not induce global DNA demethylation. Its demethylation effect is independent of DNA synthesis, and butyrate induces sporadic demethylation of certain genes, such as demethylation of the *RARβ2* promoter (54).

Selenium, an essential micro-element, has cancer-preventing potential due to its antioxidant and pro-apoptotic effect (55,56). An in vitro treatment of *Caco-2* cells with 1 or 2 μM selenite for 7 days induced global hypomethylation and promoter methylation of the *p53* gene. In addition, male Fischer 344 rats fed with a selenium-deficient diet had significantly hypomethylated liver and colon DNA compared with rats fed with 0.1 or 2.0 μg selenium/g diets for 6 wk (55). Similarly, *HT-29* cells cultured in the absence of selenium had significantly hypomethylated DNA but increased DNMT1 protein expression compared with cells cultured in the presence of 1 or 2 μmol/L selenium (55,56). In human colon cancer, selenium plays a role in chemoprevention by inhibiting DNMT (57), thus suppressing DNA methylation. Selenium is indirectly influencing plasma homocysteine

concentrations and the SAM:SAH ratio in rat models (58,59). However, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) has provided no evidence that selenium prevents prostate, lung, or colorectal cancer (60). In addition, recent findings suggest that selenium, and other dietary components, can have different effects in different species. Whereas plasma and tissue homocysteine concentrations were decreased by selenium deprivation both in CD-1 mice and Fischer-344 rats, plasma glutathione was increased only in rats (59). These findings suggest that species differences need to be considered when interpreting results from these models.

Histone Posttranslational Modifications

Histones have an active function in the regulation of chromatin structure and gene expression. Histone tails can be modified by acetylation, methylation, phosphorylation, poly-ADP ribosylation, sumoylation, or ubiquitination (2,61). The histone code characterizes a combination of histone modifications that determines the interaction of chromatin with chromatin-binding proteins (62). DNA methylation and histone modifications are not independent events. Methylation of cytosine within CpG islands is associated with binding of methyl-cytosine binding proteins (*MBPs*) and subsequent recruitment of enzymes that catalyze histone modifications (1, 2).

Histone acetylation of lysine amino group residues by histone acetyltransferases (HATs) neutralizes the positive charge on lysines and releases the histone tail from the negatively charged DNA. This change results in a relaxed chromatin structure that is more readily accessible to transcriptional factors for subsequent DNA transcription/gene expression (2) (Fig. 2). Thus, histone acetylation is associated with transcriptionally active chromatin. Histone deacetylation, catalyzed by histone deacetylases (HDACs), leads to the condensation of chromatin and suppression of DNA transcription. In the deacetylated state, the lysine amino groups are positively charged and allow the histone tails to interact tightly with the negatively charged DNA

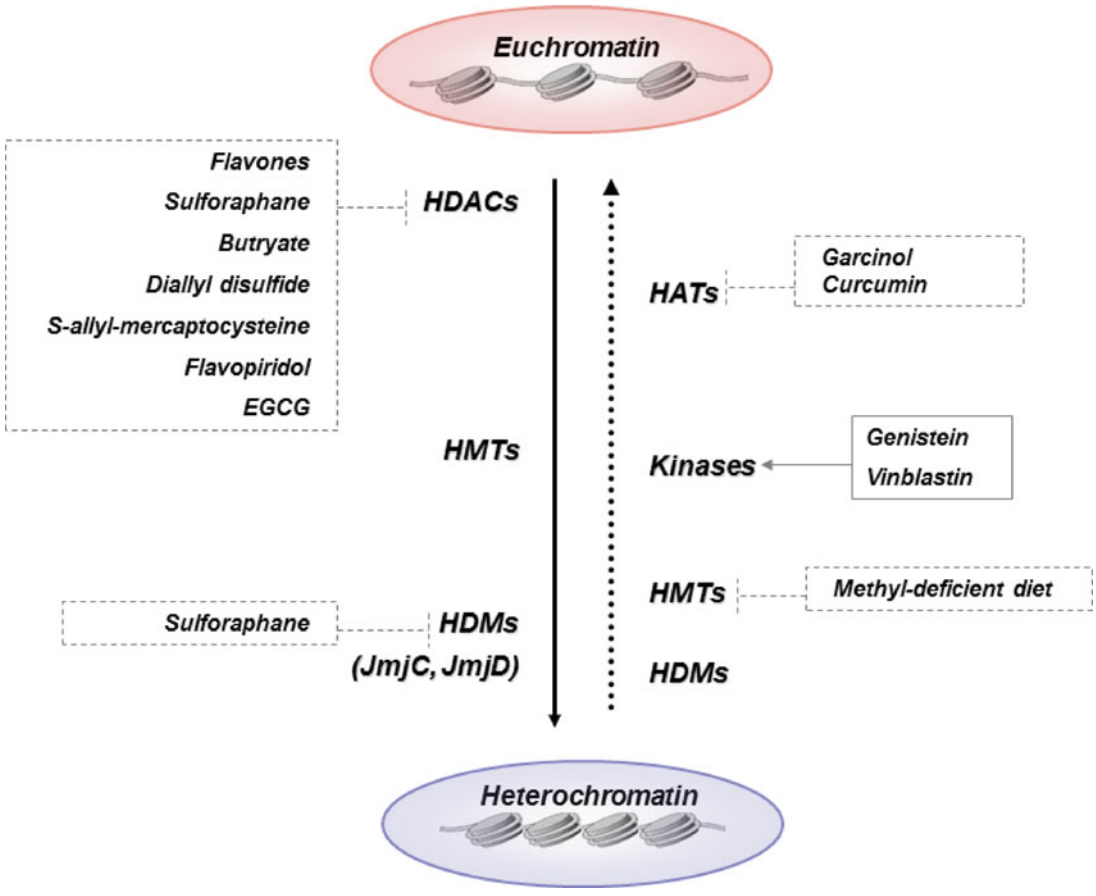

FIG. 2. Histone modifications and histone variants determine the interaction of histones with DNA and the interaction of non-histone proteins with chromatin. Histone acetylation by histone acetyltransferases (*HATs*) neutralizes the positive charge on lysines and releases the histone tail from the negatively charged DNA, by which chromatin is 'loosened' and accessible to the transcriptional factors. Histone deacetylases (*HDAC*) deacetylize lysine residues in histone tails, and nucleosomes are more tightly compacted. Histone methylation does not alter the charge of the histone tails, but influences the chemical characteristics of histones and their affinity to transcription factors or other regulatory proteins. Histone methylation is catalyzed by histone methyltransferases (*HMTs*), while demethylation is catalyzed by the histone demethylases (*HDMs*). Factors known to contribute to histone modifications alterations influencing the histone-modifying enzymes include folate, flavones, sulforaphane, butyrate, diallyl disulfide, genistein, and curcumin. (Color figure available online).

strand (61,62). Aberrant histone acetylation has been associated with cancer pathology (63).

Histone methylation of lysine (K) and arginine (R) residues of histones H3 and H4 can have activating and repressing effects on transcription, depending on the modification, the type of amino acid and its position in the histone tail (2,61). Whereas methylation of the residue of the fourth lysine on histone 3 (H3K4) leads to transcriptional activation, methylation of the ninth lysine on histone 3 (H3K9) leads to transcriptional repression. Histone methylation does not alter the charge of the histone tails but influences the chemical characteristics of histones and their affinity for transcription factors or other regulatory proteins. Histone methylation is catalyzed by histone methyltransferases, whereas the removal of methyl groups is catalyzed by histone demethylases (64) (Fig. 2).

Histone phosphorylation has an important role in mitosis, genomic stability and cell proliferation (2). The core histones and histone H1 undergo phosphorylation on specific serine and threonine residues by H1 and H3 kinases. H1 phosphorylation weakens the binding of H1 to DNA, promoting free access for transcriptional factors, thus stimulating gene expression. Aberrant histone phosphorylation has been observed in many cancers such as breast, prostate, and colorectal cancer (61).

Dietary Components and Histone Modifications in Cancer

A growing body of evidence suggests that plant-derived organic compounds can have an impact on histone modifications (Fig. 2). The potential ability of dietary compounds to reactivate epigenetically silenced genes in cancer cells may be essential for cancer prevention and therapy.

Folate can influence histone methylation in cancer. A diet low in methyl-donors leads to changes in H4-K20 trimethylation and H3-K9 acetylation, as observed during hepatocarcinogenesis (65).

EGCG induces a number of histone modifications in human melanoma cells *A431* treated with 20 μM for 6 days. EGCG treatment decreased histone deacetylase activity and increased acetylation of lysine 5, 12, and 16 on histone H4; lysine 9 and 14 on histone H3; and decreased levels of methylated H3-Lys 9 (43). Thus, EGCG has the potential to influence cancer risk through its dual actions on DNA demethylation and histone modifications, which, in turn, can induce transcriptional activation of tumor suppressor genes.

Genistein, in addition to its effect on DNA methylation, has been associated with histone modifications. Long-term genistein treatment of *MCF-7* breast cancer cell line reduces acetylation of H3 and alters growth response to mitogens and HDAC inhibitors (66). In prostate cancer cells *LNCaP* and *PC-3* (treated with 25 and 50 μM genistein for 72 h) genistein reactivated tumor suppressor genes PTEN and CYLD by modulating H3-K9 methylation and deacetylation of these genes. In addition, genistein increased acetylated H3-K9 in p53 and FOXO3a through downregulation of endogenous SIRT1-mediated deacetylation independent of promoter DNA methylation status (67). Furthermore, in prostate cell lines *LNCaP*, *DuPro*, and *RWPE,* treatment with 10 and 25 μmol/L genistein increased H3/K4 acetylation at the *p21* and *p16* transcription start sites and the expression of HATs (68). In breast cancer cell lines *MDA-MB-231* and *BT20*, treatment for 4 days with 15 and 30 μM genistein, respectively, revealed an induced H1 phosphorylation, transcriptional activation, and G2/M arrest (69).

Resveratrol antagonizes dioxin-induced histone modifications at the *BRCA-1* gene, repression of BRCA-1 protein expression and reduces dioxin-induced DNA strand breaks in breast cancer cell line *MCF-7* (48). Pretreatment with reveratrol increased acetylation of H4 and H3K9, reduced methylation of H3K9 and modulated the recruitment of MBD2 to the *BRCA-1* promoter in breast cancer *MCF-7* cells treated with tetrachlorobenzo dioxin (48). These findings indicate that epigenetic silencing of the *BRCA-1* gene might be preventable with resveratrol and provide the molecular basis for the development of cancer prevention and therapy strategies.

Curcumin treatment of brain cancer cells induced hypoacetylation of H3 and H4 histones (70). The opposite was observed in prostate cancer cells, where curcumin induced acetylation of H3 and H4, and apoptosis by the involvement of *Bcl-2* family genes and *p53* (71). These discrepancies might originate from differences in tumor type and tumor models but could also be due to time, cell-type, and dose-dependent effects of curcumin. All these issues require further investigation.

Quercetin induced a significant tumor growth delay in 7,12-dimethylbenz anthracene induced hamster buccal pouch carcinomas (50). This effect was attributed to induction of cell cycle arrest and apoptosis that correlated with inhibition of HDAC-1 (50).

Butyrate is an HDAC inhibitor and promotes acetylation of histones, leading to expression of genes involved in cellular differentiation and apoptosis in several cancer models (72). In addition, butyrate increases histone phosphorylation of *ERK* in *HT29* colon cancer cells (53). In a mouse model of dimethyl hydrazine-induced colorectal cancer, sodium butyrate alone, or synergistically with folic acid, significantly reduced the incidence of cancer, downregulated histone H3 acetylation and *p21* gene expression (73).

Sulforaphane, an isothiocyanate from cruciferous vegetables and broccoli, inhibits HDAC activity in human colon, prostate, and breast cancer cells (72,74,75). In vitro, in *BPH-1*, *LnCaP*, and *PC-3* prostate cancer cells, sulforaphane inhibited HDAC activity (after 48-h treatment with 15 μM sulforaphane). This was accompanied by the increase of p21 and Bax protein levels, cell cycle arrest and activation of apoptosis (76). Sulforaphane inhibited proliferation and induced apoptosis in *MCF-7* and *MDA-MB-231* breast cancer cells in dose- and time-dependent manner (77). In addition, cell growth was completely inhibited after 6 days of treatment with 15 μM and 20 μM of sulforaphane by inhibiting DNMTs, demethylating the *hTERT* promoter, and increasing active chromatin marks (acetyl-H3, acetyl-H3K9 and acetyl-H4), while decreasing inactive

chromatin marks (trimethyl-H3K9 and trimethyl-H3K27) (77). In in vivo model, HDAC activity was inhibited significantly in the colonic mucosa, prostate, and peripheral blood mononuclear cells of Apc-minus mice after a single oral dose of 10 μmol sulforaphane (78). The protective effect of broccoli was observed in smokers and nonsmokers submitted to a controlled broccoli diet, which was associated with a significant decrease in DNA strand breaks (79,80). In healthy human subjects, a single ingestion of 68 g (1 cup) of broccoli sprouts inhibited HDAC activity in circulating peripheral blood mononuclear cells 3–6 h after consumption. Although the HDAC activity returned to normal by 24 h, histone hyperacetylation was evident for at least 48 h (81). This was the first study to show that natural dietary compounds from broccoli have a substantial effect on HDAC activity in humans (81).

S-allyl-mercaptocysteine, an organosulphur compound found in garlic, acts as an HDAC inhibitor and induces rapid and persistent histone H3 and H4 hyperacetylation in human cancer cells (75,82). Allyl mercaptan, a garlic-derived organosulfur compound, in the doses of 2, 20, and 200 μM shows dose-dependent inhibition of histone deacetylase and enhanced Sp3 binding on the *P21WAF1* promoter, followed by the subsequent recruitment of *p53* (83).

Diallyl disulfide (DADS) is a natural HDAC inhibitor present in garlic and other *Allium* vegetables (84). It has been shown that treatment with 200 μM DADS for 6 h in human colon cell lines *HT-29* and *Caco-2* inhibits cell proliferation (85). DADS exerted this effect through HDAC inhibition, histone hyperacetylation, and an increase of *p21* expression (85). Interestingly, a single administration of DADS has a transient effect on histone H3K14 acetylation, while repetitive treatment with DADS results in a prolonged hyperacetylation of histone H3 (86). These findings indicate that not only a dose, but the pattern of treatment can influence the response to bioactive dietary compounds.

RNA-Associated Silencing by Micro RNAs in Cancer

Micro RNAs (miRNAs) are small, 18–26 nucleotide long, non-coding RNAs that have a role in post-transcriptional regulation, by binding to the 3' untranslated region (3'UTR) of target messenger RNA (mRNA) (87,88). A miRNA acts either by the complete complementary base pairing, that results in mRNA degradation, or by a partial base pairing, which leads to translational inhibition of the targeted mRNA (87,88) (Fig. 3). In addition, miRNAs can exert their activity through transcriptional regulation. It has recently been shown that miRNAs can bind complementary sequences in the genome and induce gene silencing by recruiting repressive proteins and inducing repressive chromatin marks (89,90). MiRNAs regulate cell proliferation, differentiation and apoptosis and changes in miRNA expression are common events in cancer. Down-regulation of subsets

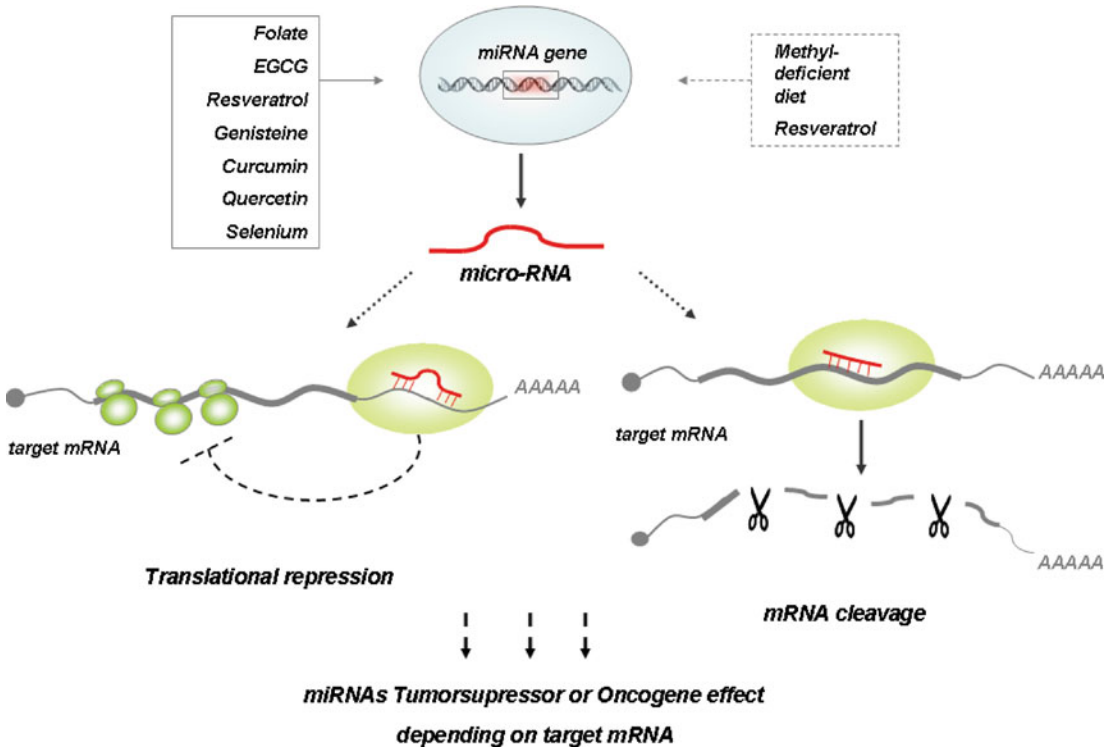

FIG. 3. Micro-RNAs (*miRNAs*) are small (an average of 22 nucleotides), non-coding RNAs that have a role in posttranscriptional regulation, by binding to the target messenger RNA (mRNA). Micro-RNAs function as transcriptional regulators either by complete complimentary base pairing that result in the degradation of the mRNA or by partial base pairing, which leads to translational inhibition of the targeted mRNA. Factors known to contribute to the altered *miRNAs* gene expression includes dietary compounds, such as folate, EGCG, genistein, quercetin, curcumin and resveratrol. (Color figure available online).

of miRNAs in cancers suggests that some of them may act as putative tumor-suppressor genes, while up-regulation suggests that many miRNAs may act as oncogenes, depending on their targets (87,88).

Dietary Components and miRNA Changes in Cancer

Recent evidence suggests that diet, lifestyle, and genetic factors can affect cancer risk through modulation of miRNAs (Fig. 3). For example, maternal high fat diet prior to conception, during pregnancy and lactation induced long-lasting changes in *IGF2* expression and several key miRNAs in the offspring of mice (7).

Folate, along with an important role in DNA methylation, can influence miRNA expression. Folate deficiency induced a pronounced global increase in miRNA expression with miRNA-222 showing significant overexpression in vitro and in vivo in human lymphoblast cells (91). Rats fed a methyl-deficient diet developed hepatocellular carcinoma at 54 weeks of age with a tumor-specific decrease in *miRNA-122*, without carcinogen treatment. Conversely, a methyl-adequate diet reversed this effect and prevented tumor-specific miRNA alterations and cancer development (92). Hepatocarcinogenesis induced by methyl deficiency in rats led to profound downregulation of miRNAs (93,94), including *miRNA-34a* and *miRNA-127*, involved in the regulation of apoptosis and cell proliferation, respectively (95). In addition, folic acid supplementation suppressed *miRNA-10a* expression induced by alcohol exposure (96), which suggests that folate could exert a protective role in cancer through modulation of *miRNA* expression.

EGCG treatment modified expression of a number of miRNAs in human hepatocellular carcinoma cell line, including *miRNA-16*, known to target the anti-apoptotic protein *Bcl-2*. Transfection with anti-*miRNA-16* inhibitor counteracted the EGCG effect on *Bcl-2* down-regulation in hepatocellular cell line (97).

Genistein treatment with 200 μM for 36 h significantly decreased cell proliferation by $\sim$60% in the human uveal melanoma cell line *C918* and induced dose-dependent expression of *miRNA*-27a (98). In vivo, impact of genistein was examined in BALB/C nu/nu mice injected with 25, 50, and 100 mg/kg body weight/day genistein intraperitoneally for 1 mo. These experiments revealed that genistein significantly inhibits the uveal melanoma xenograft growth in vivo (98). Another study reported genistein effects on miRNA-16 expression and synergistic induction of apoptosis in a malignant *B-1* cell line used as a murine chronic lymphocytic leukemia model (99). This suggests that genistein can potentially modulate the biological effects of miRNAs. After genistein treatment of ovarian cancer cell lines *UL-3A* and *UL-3B*, the number of miRNAs was differentially expressed and associated with the induction of ER-α and ER-β and with a significant decrease in migration and invasion (100). A recent study showed that genistein treatment up-regulated tumor suppressor *ARHI* by downregulating *miRNA-221* and *miRNA-222* in prostate cancer cell line *PC-3* (101).

Resveratrol treatment of human *SW480* colon cancer cells, decreased levels of several oncogenic miRNAs that target *PDCD4* or *PTEN* (54). Simultaneously, the treatment increased the tumor suppressor *miR-663,* known to target transforming growth factor (TGF) β1 transcripts (102).

Curcumin treatment with 10 μmol/L of human pancreatic cancer cell line *BxPC-3* showed an altered miRNA expression profile. The treatment led to upregulation of *miRNA-22* and downregulation of *miRNA-199a* (103). In addition, curcumin reduced *Bcl-2* expression by up-regulating *miRNA-15a* and *miRNA-16* in *MCF-7* breast cancer cell line (104), thereby inducing apoptosis.

Quercetin treatment induced *miR-146a,* a negative regulator of pro-inflammatory NF-κB activation, in human colon cells *HT-29* (84).

Selenium treatment with 2.5 μM selenite, a natural form of selenium, in prostate cancer cells *LNCaP* induced *p53*-mediated apoptosis in a time-dependent manner and induced expression of *miR-34,* the transcriptional target of p53 (105).

Further studies are needed to establish the optimal dose and the duration necessary for a chemopreventive effect of these bioactive food components. In addition, tissue specificity and species differences need to be considered when interpreting results from various models, from cancer cells, mouse models, to human subjects.

CONCLUSIONS AND PERSPECTIVES

Recent studies provide substantial evidence that dietary components can have an important role in cancer prevention through epigenetic mechanisms. However, the precise mechanisms whereby bioactive dietary components alter epigenetic changes and their cellular targets in human cancers are largely unknown. Current literature strongly suggests that dietary components of fruits, vegetables, and various other plants may affect DNA methylation, histone modifications and miRNA expression in cancer. However, the protective effect is unlikely to be due to a single dietary component. Thus, the identification of relevant compounds and metabolites is needed. Another key issue is the ability of bioactive dietary components to reach the target tissues in concentrations that are sufficient to induce desirable epigenetic changes. In relation to that, metabolism can play a pivotal role in both affecting the concentrations of bioactive components and generating intermediates with the potential to induce epigenetic changes. Moreover, complex interactions between dietary components, metabolism, and environmental factors may considerably complicate studies aiming at identifying components which might induce or prevent cancer development. In addition, eating patterns could play a more important role in modulating cancer risk than consumption of any specific food or nutrient, and this may be the key approach for cancer prevention.

Epigenetic modifications are tissue-specific and play an important role in cell differentiation. Therefore, bioactive food components may induce different epigenetic changes in

different tissues and even in different cell types of the same tissue. In addition, epigenetic changes induced by bioactive dietary components can exert temporal associations. Therefore, it is crucial to characterize tissue- and cell-specific effects of bioactive dietary compounds and their kinetics.

An emerging question is whether natural DNMT and HDAC inhibitors have only beneficial effects or if there are conditions when they might be harmful. Natural DNMT inhibitors might cause unselective changes and global genome DNA hypomethylation, which may activate repetitive elements to affect genome stability, or activate transcription of latent viruses or oncogenes to promote malignant transformation. Similarly, dietary components that affect histone modification could have substantial effects on target genes that encode proteins involved in cell cycle regulation, proliferation, metabolism, and signal transduction. Further studies are needed to determine effective doses and concentrations of bioactive food components relevant for cancer prevention or treatment. Bioactive dietary compounds are effective at low micromolar concentrations in vitro and in vivo. Conversely, DNMTs and HDAC inhibitors used therapeutically are effective at higher doses, with substantial toxicity and drug resistance effect. Additional research is necessary to determine beneficial versus deleterious responses in healthy individuals, as well as individuals with different stages of cancers.

Another question is the critical times for exposure during fetal development, throughout a lifespan and during ageing. It has been shown that maternal diet and folate intake can affect the long-term DNA methylation changes that occur later in life of their offspring.

Developing relevant animal and tissue culture models for the studies of dietary and environmental impact on epigenetic changes will be essential for elucidating their relationship and potential interactions. In addition, species differences need to be considered when interpreting results from cancer cells, mouse models and human subjects. An important area for future research is to develop the methods for genome-wide analysis and high-throughput assays for further elucidation of the complex interplay between DNA methylation, histone modifications and miRNAs.

The complex interactions among environmental, genetic, and epigenetic factors during cancer development have not yet been fully identified. Elucidating the epigenetic mechanisms that underlie these modifications may serve as a tool to predict the individuals' genetic susceptibility to cancer, provide dietary recommendations, or provide therapeutic applications of natural compounds against cancer.

Taken together, an increasing number of studies support a role of diet in cancer prevention and treatment. Nevertheless, to provide safe dietary recommendations, it is necessary to define the bioactive dietary components and to establish the optimal doses required for a chemopreventative effect. Therefore, further studies that aim at elucidating mechanisms of epigenetic changes and their cell-type specificity and temporal patterns are necessary. Moreover, the influence of genetic and environmental factors on protective epigenetic changes induced by bioactive dietary components need to be established. However, despite many unresolved questions, there is a promising future for dietary recommendations in cancer prevention and for therapeutic applications of natural dietary components in the future treatment of cancer.

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