Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention

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Abstract

The growing interest in cancer epigenetics is largely due to the reversible nature of epigenetic changes which tend to alter during the course of carcinogenesis. Major epigenetic changes including DNA methylation, chromatin modifications and miRNA regulation play important roles in tumorigenic process. There are several epigenetically active synthetic molecules such as DNA methyltransferase (DNMTs) and histone deacetylases (HDACs) inhibitors, which are either approved or, are under clinical trials for the treatment of various cancers. However, most of the synthetic inhibitors have shown adverse side effects, narrow in their specificity and also expensive. Hence, bioactive phytochemicals, which are widely available with lesser toxic effects, have been tested for their role in epigenetic modulatory activities in gene regulation for cancer prevention and therapy. Encouragingly, many bioactive phytochemicals potentially altered the expression of key tumor suppressor genes, tumor promoter genes and oncogenes through modulation of DNA methylation and chromatin modification in cancer. These bioactive phytochemicals either alone or in combination with other phytochemicals showed promising results against various cancers. Here, we summarize and discuss the role of some commonly investigated phytochemicals and their epigenetic targets that are of particular interest in cancer prevention and cancer therapy.

Keywords

Epigenetics; phytochemicals; DNA methylation; histone deacetylation; cancer

1. Introduction

Carcinogenesis is considered to be the outcome of deregulated genetic and epigenetic events. Epigenetic alterations are important as they link the behavior of cells to their environmental interactions and thus determine the susceptibility of a cell to transforming changes. As these changes do not involve alterations in the genome constructs, these epigenetic events occur constantly during the life of the cells. DNA methylation, histone tail modifications, chromatin remodelling and miRNA-mediated multi-gene silencing are considered to be the major epigenetic changes that are involved in maintaining cellular homeostasis and differentiation states. Multiple studies have revealed that global DNA hypomethylation events are a major characteristic of most of types of cancer and contribute to genomic instability by activating retrotransposons and other silent genomic regions [1]. On the other hand, promoter hypermethylation events occur in important tumor suppressor genes, indicating that it is inappropriate DNA methylation that is important in driving the process of carcinogenesis [2]. Histone modifications also occur, including acetylation and methylation of lysine residues, methylation of arginine residues, phosphorylation of serine or threonine residues, ubiquitination and sumoylation of lysine residues, ADP ribosylation of glutamic acid residues and isomerisation of proline residues, *etc.*. These modifications define the patterns of chromatin remodelling, thus determining the resultant gene expression and gene silencing patterns. The microRNAs (miRNAs), which are recently identified non-coding RNAs, also have been shown to contain gene-expression regulatory activities and are capable of functioning both to suppress and promote oncogenesis. Deregulated miRNA transcription leads to upregulation of oncogenes and silencing of tumor suppressor genes in lung, breast, head and neck as well as bone cancers [3–6].

Dietary phytochemicals or supplements are not only a rich source of minerals, vitamins and micronutrients but also contain bioactive components such as anti-oxidants, polyphenols and alkaloids, which are more than the basic nutrients. These bioactive compounds have shown great potential against many diseases including cancers through genetic and epigenetic modifications [7–10]. In this review article, we focus on the major types of epigenetic modifications, such as DNA methylation, histone modifications and miRNA-mediated gene silencing in cancer progression, and epigenetic targeting by phytochemicals in cancer prevention and therapy.

2. DNA methylation

Epigenetics is defined as the study of heritable but reversible changes in gene expression that occur without alterations in the sequences of underlying DNA. Epigenetic modifications often alter gene expression and, in particular, expression of the tumor suppressor, promoter, and oncogenes that are crucial for cellular proliferation, differentiation and survival during carcinogenesis. Among epigenetic modifications, DNA methylation is the best studied modification of DNA [11]. S-adenosyl methionine (SAM) functions as a universal methyl

group donor in the methyl transfer reactions catalyzed by DNA methyltransferases (DNMTs) in the eukaryotic nucleus. There are two types of DNMTs present in eukaryotes including maintenance methyltransferase (DNMT1) and the *de novo* methyltransferases (DNMT3A and DNMT3B). DNMT1 functions in maintaining the pre-established patterns of DNA methylation, while the *de novo* enzymes establish new patterns of methylation in the fully un-methylated DNA. DNA hypermethylation of tumor suppressor genes is a rather frequent event in most of the cancers both during the initiation or the progression events [2]. Gene hypermethylation also might initiate recruitment of the methylation-dependent DNA binding proteins (MBDs) to the hypermethylated DNA sites. The MBDs further help in silencing of methylated genes by recruiting repressor complexes to these regions. These proteins are commonly found occupying the hypermethylated gene promoters in multiple cancers [12]. In addition to the MBDs, a transcriptional domain in DNMT1 also recruits histone deacetylase (HDACs) and other chromatin re-modelling proteins to the target sites that can modify acetylation and methylation status of histones, thereby inhibiting transcriptional access to the chromatin [13]. An example of such aberrant methylation-mediated gene silencing was demonstrated in ultraviolet-B (UVB) radiation-induced skin tumors in the SKH-1 mouse model. This study clearly demonstrated the positive correlation between transcriptional repression of tumor suppressor $p16^{INK4a}$ and *RASSF1A* genes and the UVB-mediated hypermethylation and subsequent recruitment of MeCP2 and MBDs at gene regulatory regions in skin cancer model *in vivo* [14]. MeCP2 is the founding member of the MBD family of transcriptional repressors, which creates a repressive environment at the target DNA site through recruitment of HDAC-containing transcriptional repressor complexes to the methylated DNA [15]. In addition, MeCP2 is also linked with higher H3K9 methylation, which is an important heterochromatin mark [16]. Hence, DNMTs inhibitors are important in cancer therapy and some FDA-approved inhibitors of DNMTs, such as 5-azacytidine and 5-aza-2′-deoxycytidine, are already being used as therapeutic drugs against multiple cancer types [17, 18]. Many of the synthetic inhibitors have, however, been shown to cause adverse toxic effects and are narrow in their specificity. Hence, phytochemicals, which are widely available and have lesser side effects or toxicities, are being tested for their role in direct or indirect inhibition of DNMTs activity in cancer prevention and therapy. DNMTs-mediated differential effects on promoter methylation and histone acetylation on gene regulation by bioactive phytochemicals are depicted in Figure 1.

3. Histone modifications and chromatin remodelling

Eukaryotic DNA is organized in a complex structure known as chromatin, which is comprised of DNA, histones and several other DNA-binding proteins. In addition to promoting a compact structure, chromatin organization also helps in the regulation of gene expression by restricting the access of different DNA binding proteins or protein complexes to the genetic material. The processes of "opening up" of chromatin and its compaction are associated with a number of ATP-dependent multi-enzyme complexes known as chromatin remodelling complexes. The chromatin remodelling is triggered by various histone tail modifications, which determine the state of activity of chromatin. The best studied histone modification, lysine acetylation, leads to opening up of the chromatin due to the negative charge conferred by the acetyl moieties, which reduces the histone-DNA interactions. The

lysine acetylation reactions are catalyzed by histone acetyltransferases (HATs), which transfer the acetyl groups from acetyl coenzyme A to the lysine moieties in the nucleosomes. HATs are classified into three families, the GCN5 N-acetyltransferase (GNAT), the MOZ/YBF2/SAS2/TIP60 (MYST) and the p300/CBP families [19, 20]. HATs also play important roles in regulating cell cycle regulatory protein expression and also can bind directly to the cell cycle regulatory apparatus [21].

Histone deacetylases (HDACs) remove the acetyl groups from lysine residues to reduce the negative charge thus leading to chromatin compaction. Four distinct classes of HDACs in humans have been identified based on their structural similarity to yeast proteins as well as their localization and acetylation activities [20]. Class I, II and IV HDACs are zinc-dependent histone deacetylases, while class III HDACs, also known as sirtuins, are NAD^+-dependent HDACs. Different HDACs function in distinct ways and on different downstream targets leading to their diverse tumor suppressor and oncogenic activities. Overexpression and altered activities of HDACs are associated generally with the silencing of tumor suppressor genes, epithelial to mesenchymal transitions and metastasis [22–24]. Over-expression of *HDAC1* resulted in downregulation of *p53* and *von Hippel-Lindau* tumor suppressor genes and stimulated angiogenesis of human endothelial cells [22], while HDAC10 suppresses metastasis of cervical cancer through inhibition of matrix metalloproteinases 2 and 9 expression [25].

In general, histone acetylation is associated with gene activation and is abundant in the euchromatin whereas deacetylation is linked to gene repression and occurs in the heterochromatin. Nevertheless, gene repression or activation is not completely dependent on histone acetylation or methylation, but rather is dependent on the site and degree of methylation or acetylation on histone tails. Some of the active chromatin markers associated with gene expression are histone methylation on histone H3 at lysine 4 (H3K4), on histone H3 at lysine 36 (H3K36), on histone H3 at lysine 79 (H3K79) and on histone H4 at lysine 20 (H4K20); while the inactivation markers associated with gene repression are methylation on histone H3 at lysine 9 (H3K9) and on histone H3 at lysine 27 (H3K27) [26]. It is recognized widely that HDACs are promising targets for cancer prevention and therapy. Some of the well-studied HDAC inhibitors are trapoxin (TPX), trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA). Among them, Vorinostat or SAHA and Romidepsin (Istotax) are commercially-available FDA-approved HDAC inhibitors for treatment of cutaneous T-cell lymphoma [27]. Some other HDAC inhibitors such as Panobinostat, valproic acid and Belinostat are in different phases of clinical trials.

4. Dietary phytochemicals and their epigenetic modulatory activities

Enthusiasm for the use of dietary phytochemicals in the prevention and therapy of different diseases has increased in recent years. Possible reasons behind this interest lie in their natural origin, widespread availability, lesser side-effects and the possibility of inclusion in the routine diet. Traditionally, these phytochemicals have been utilized in the treatment of various diseases since ancient times. There now has been a tremendous increase in the knowledge concerning their mechanisms of actions and molecular targets. Interestingly, these dietary factors have shown a capability to regulate the patterns of expression of

multiple genes through epigenetic modulatory mechanisms. Herein, we provide an overview in which the epigenetic mechanisms of action of dietary component in different tumor models are summarized. It should be noted, however, that the mechanisms of action of dietary components vary among various cancer types.

Polyphenols are one of the essential bioactive dietary supplements largely present in fruits, vegetables, seeds and nuts. There are approximately 8000 polyphenols present in the diet and these can be classified into ten different generalized classes according to their chemical structure [10]. The major classifications are catechins/epicatechins, stilbenes, benzoquinones, acetophenones, flavonoids, phenolic acids, proanthocyanidins, ellagitannins and anthocyanins [28]. Among these, the most commonly studied polyphenols are (−)-epigallocatechin-3-gallate (EGCG; found in green tea), resveratrol (present in grape skin) and curcumin (found in turmeric). These dietary polyphenols have been shown to have chemopreventive and therapeutic potential in preclinical models against various cancers [7, 29, 30]. Significant progress has been made in understanding the effects of these agents in altering signaling cascades and other inflammatory pathways involved in carcinogenesis. Further, these dietary polyphenols have shown to reverse the epigenetic changes occurred during the process of carcinogenesis.

4.1. Tea catechins/epicatechins

The tea plant (*Camellia sinensis*) is cultivated in more than 30 countries. Green, black, and oolong teas are the most common varieties of tea all of which are derived from the leaves of the tea plant defined but differ according to their manufacturing processes [31, 32]. Studies have shown that green tea possesses significant beneficial effects due to an abundance of monomeric catechins or epicatechins, which include (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) [32]. Of these, EGCG is the major and most active ingredient of green tea polyphenols (GTPs) and is shown to have potent anti-cancer activities both *in vitro* and in *in vivo* models [33, 34]. In particular, EGCG has been shown to inhibit cellular proliferation and to induce apoptosis in many cancer cell types through multiple mechanisms [35–37]. Several elegant studies have addressed the modes of actions of various tea polyphenols, including EGCG, in cancers. Recent studies have suggested that the anti-cancer activity of EGCG is mediated, at least in part, through its epigenetic modulatory activities, such as inhibition of DNMTs and HATs [33, 38, 39].

EGCG is involved in direct inhibition of DNMTs by forming hydrogen bonds in their active sites that hinder substrate binding [39]. EGCG also has been reported to reduce the available S-adenosyl-L-methionine (SAM), a methyl donor for DNMTs, and induce S-adenosyl-L-homocysteine (SAH), a potent inhibitor of DNMTs, in a mechanism in which EGCG mediates indirect inhibition of DNMTs [27]. EGCG-mediated indirect inhibition of SAM (but not SAH) has been demonstrated convincingly by both *in vitro* and *in vivo* studies [39, 40]. The inhibition of DNMTs can lead to reversal of the silencing of tumor suppressor genes in cancer cells, which has been found to be one of the effective treatment strategies against cancer. Fang *et al.* [39] showed that the treatment of EGCG in human oesophageal KYSE 510 and 150 cells leads to a concentration- and time-dependent reversal of

hypermethylation and re-expression of several known tumor suppressor genes such as $p16^{INK4a}$, O^6-methylguanine methyltransferase (*MGMT*), human *mutL* homologue 1 (*hMLH1*) and retinoic acid receptor β (*RAR*β) [39]. Similarly, we demonstrated that the topical application of EGCG, which is protective against ultraviolet-B (UVB) radiation responses, restores UVB radiation-induced global hypomethylation in the SKH-1 hairless mouse model [41]. We also further demonstrated that treatment of cancer cells with EGCG not only reactivates tumor suppressor genes but also leads to down-regulation of *human telomerase reverse transcriptase (hTERT)*, a catalytic subunit of telomerase, a tumor promoter and aging-related gene [42, 43]. In contrast to tumor suppressor genes, demethylation of the *hTERT* promoter is, in general, associated with its repression. This paradox is explained partially by the observation that the EGCG-mediated demethylation recruits gene repressor complexes to the *hTERT* promoter resulting in its transcriptional downregulation [43]. In addition, *Wnt*, an oncogenic ligand family, has been reported to be downregulated by EGCG-mediated promoter demethylation in lung cancer cells [44].

These studies suggested that the inhibition of tumor promoter genes and induction of tumor suppressor genes are of central importance in cancer prevention and therapy. In accordance, Meeran *et al* reported that EGCG and a pro-form of EGCG inhibit *hTERT* expression by inducing gene-specific demethylation and chromatin modifications in human breast cancer cells [38]. The authors demonstrated that EGCG and the pro-form of EGCG induced chromatin alterations that facilitated the binding of many *hTERT* repressors such as MAD1 and E2F-1 to the *hTERT* regulatory region, thereby contributing to their transcriptional repression [38]. In yet another study, we demonstrated that the treatment of A431 skin cancer cells with EGCG downregulates the expression and activities of DNMTs and the level of 5-methylcytosine resulting in the re-expression of key tumor suppressor genes such as $p16^{INK4a}$ and $p21^{CIP1/WAF1}$ [33]. In addition to DNA methylation, we demonstrated that the treatment of EGCG decreased HDAC activity and increased levels of active chromatin markers, such as acetylated lysine 9 and 14 on histone H3 and acetylated lysine 5, 12 and 16 on histone H4, which also contributed to the re-expression of tumor suppressor genes in A431 skin cancer cells thereby enabling their inhibition of cellular proliferation and induction of apoptosis [33]. In another study using oral carcinoma cells, partial demethylation of *RECK*, a tumor suppressor gene, by EGCG was found to be well correlated with inhibition of tumor invasion, angiogenesis and metastasis [45]. Furthermore, EGCG has been demonstrated to inhibit the invasive potential of human pancreatic adenocarcinoma cells by modulation of HDAC activity [46] and EGCG has been shown to induce the degradation of DNMT3A and HDAC3 in methylation-sensitive colon cancer cells, in part, through inhibition of E3 ubiquitin ligase [47]. EGCG also was found to induce tissue inhibitor of matrix metalloproteinase-3 (TIMP-3) gene, which negatively regulates matrix metalloproteinases (MMPs) activity, through the enrichment of H3K27 trimethylation in the promoter and a concomitant increase in histone H3K9/18 acetylation in breast cancer cells [48].

Combination of HDAC inhibitors with EGCG (a known DNMT inhibitor) is another effective approach for cancer prevention and therapy. Studies conducted by Li *et al.* [49] showed that a combination of EGCG with an HDAC inhibitor reactivates estrogen receptor-

α (ERα) expression in ERα-negative human breast cancer cells through a process associated with chromatin modifications. EGCG in combination with TSA, a HDAC inhibitor, synergistically reactivates *p16*[INK4a] expression, in part, through a reduction of promoter expression in CA46 human lymphoma cells [50]. Similarly, EGCG in combination with sodium butyrate synergistically inhibits cell cycle arrest and induces cellular apoptosis through down regulation of HDAC1, DNMT1 and inhibition of HDAC activities in colon cancer cells [51]. Not only synthetic inhibitors, but also the dietary combination of green tea polyphenol (GTPs), a DNMT inhibitor, with sulforaphane (SFN), a HDAC inhibitor, reactivate ERα expression in ERα-negative human breast cancer cells and this was found to correlate consistently with ERα promoter hypomethylation and hyperacetylation [52]. These reactivations are a promising strategy for the effective treatment of hormonal refractory breast cancer with available anti-estrogen drugs like tamoxifen. In addition to ERα reactivation, GTPs and SFN combinations also tend to enhance cisplatin-induced apoptosis and G2/M phase cell cycle arrest through upregulation of *p21*[CIP1/WAF1], thereby enhancing the efficacy of cisplatin on both cisplatin-sensitive and cisplatin-resistant ovarian cancer cells [53]. Taken together, these data suggest that GTPs alone or in combination with dietary HDAC inhibitors can modulate epigenetic regulation of gene expression and can play a vital role in cancer chemoprevention and therapy.

4.2. Sulforaphane

Sulforaphane (SFN) is one of the major isothiocyanates present in many cruciferous vegetables and has been shown to have anti-cancer activities in several cancer models [54–57]. Although SFN has been shown to mediate anti-cancer activity through several mechanisms, including cell cycle arrest, induction of cellular apoptosis and phase-2 detoxification enzymes, there is a growing interest in their HDAC inhibitory activity [55, 56, 58, 59]. As aforementioned, HDACs are often upregulated in cancers and HDACs inhibitors play a major role in cancer prevention and therapy. SFN-mediated inhibition of DNMTs and histone methylations also have been found to play a major role in the alterations of gene expressions found in cancer prevention studies. In accordance, SFN-mediated downregulation of DNMTs also is associated positively with *hTERT* promoter demethylation, which is followed by binding of CTCF, a repressor protein, to the *hTERT* gene regulatory region in human breast cancer cells [9, 60]. Further, SFN-mediated transcriptional repression of *hTERT* correlates positively with its inhibition of cellular proliferation and induction of apoptosis in human breast cancer cells [60].

SFN also has been shown to inhibit DNMT1 and DNMT3b in human prostate cancer cells. Interestingly, SFN-induced demethylation at cyclin D2 promoter regions corresponded to an increase in cyclin D2 transcript levels, thereby exerting anti-proliferative effects on prostate cancer cells [61]. In addition, SFN treatment led to demethylation of the first five CpGs in the promoter region of the *Nrf2* gene in TRAMP C1 cells, suggesting a modulatory role on anti-oxidative stress pathways [62]. SFN enhanced the nuclear translocation of Nrf2 and increased the mRNA and protein levels of the Nrf2 target genes in 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin epidermal JB6 (JB6 P+) cells [63]. Furthermore, SFN reduced the expression of DNMTs and downregulated the expression of HDAC1, HDAC2, HDAC3 and HDAC4, thereby reactivating Nrf2, a transcription factor for

anti-oxidant enzymes [63]. A recent study by Wong *et al.* [64] evaluated genome-wide effects of SFN on promoter methylation in normal prostate epithelial cells and two prostate cancer cell lines and found that the cancer cell lines showed widespread changes in promoter methylation patterns, including both increased and decreased methylation. Although SFN has broad and complex effects on DNA methylation profiles in both normal and cancerous prostate epithelial cells the gene targets were similar within a single cell line.

4.3. Curcumin

The anti-inflammatory, anti-septic, wound-healing, anti-oxidant, anti-angiogenic and anti-cancer activities of turmeric (*Curcuma longa*) have been attributed to the yellow pigment curcumin, which is a diferuloylmethane polyphenolic compound. Multiple epigenetic activities of curcumin have been reported to date. Curcumin treatment has been shown to induce global hypomethylation in leukemia cells, further strengthening the notion of curcumin-mediated inhibition of DNMT activity [65]. Curcumin also has been shown to mediate inhibition of HDACs and HATs activity in multiple *in vitro* cancer models [65]. Significant inhibition in the expression of HDACs 1, 3 and 8, as well as of HAT p300, were found after curcumin treatment leading to repression of NF-κB and Notch 1 in Raji cells, an *in vitro* model of Burkitt's lymphoma [66]. Curcumin also has been reported to alter HDAC2 expression by chemically preventing its degradation in human monocytes [67]. The α, β-unsaturated carbonyl groups in the side-chain of curcumin are considered to be structurally important for its HAT inhibitory activity [68]. Curcumin also was found to promote proteasomal degradation of p300 and related HATs in prostate cancer cells and peripheral blood lymphocytes [68]. Recently, curcumin was found to reduce the acetylation of histone H3 in the IL-6 promoter leading to its decreased expression in rheumatoid arthritis synovial fibroblasts [69]. A number of investigators have reported curcumin-mediated alterations in miRNA expression profiles. Treatment of human pancreatic cancer cells with curcumin led to upregulated expression of 11 miRNAs, with miR-22 as the most highly overexpressed miRNA, as well as the downregulation of 18 miRNAs, with miR-199a being the most significantly downregulated miRNA [70]. Curcumin also has been shown to reduce EZH2 expression and to induce the expression of several tumor suppressor miRNAs including the let-7 family members, miR-26a, miR-146a, miR-101, miR-200b/c, *etc.* [71]. Curcumin altered the expression of the tumor suppressor miRNA, miR-203, in a panel of bladder cancer cell lines [72]. These results suggest that curcumin is a very important epigenetically bioactive compound with multiple epigenetic modifying capabilities.

4.4. Genistein

Genistein (4′,5,7-trihydroxyisoflavone), an isoflavonoid, is found in different varieties of beans and is especially abundant in soy beans. This bioactive natural compound is the most widely studied among the flavonoid group of compounds and is well known for its anti-cancer and anti-angiogenic properties. The role of genistein as a chemopreventive phytoestrogen against carcinogenesis is well established [73]. This compound is a strong anti-oxidant and a potent tyrosine kinase inhibitor. Other important mechanisms through which genistein exert its anti-cancer effects includes prevention of mutations in DNA strands; inhibition of cancer cell proliferation and angiogenesis; and proapoptotic effects [74–76]. Genistein has been found to be capable of modulating important epigenetic events,

such as DNA methylation and histone tail modifications [77–79]. The inhibitory effect on DNMTs and histone modifying activities of genistein has been established in many similar reports [80–82]. Reactivation of the tumor suppressor genes $p21^{CIP1/WAF1}$, $RAR\beta$ and $MGMT$ and $p16^{INK4a}$ through promoter hypomethylation and active chromatin modifications after genistein treatment in breast cancer, squamous cell carcinoma and prostate cancer cells led to cell cycle arrest and cell death [77, 79]. Genistein also causes the epigenetic re-expression of another tumor suppressor gene *BTG3* by altering the promoter methylation and enrichment of methyl-CpG-binding domain 2 (MeCP2) in three different renal carcinoma cell lines. In addition, genistein treatment induces the HATs activity resulting in higher enrichment of acetyl H3 and acetyl H4 as well as increased enrichment of dimethyl-H3K4 and trimethyl-H3K4 at the *BTG3* promoter [83]. Genistein, in combination with other DNMT or HDACs inhibitors, has shown synergistic epigenetic reactivation of hypermethylated tumor suppressor genes [78, 84, 85]. It alas inhibits the expression of DNMT1, DNMT3a and DNMT3b and increases the enrichment of inactivation chromatin marker trimethyl-H3K9 leading to inhibition of tumor promoter *hTERT*, the catalytic subunit of telomerase in human breast cancer cells [78].

In addition to its epigenetic effects that have been demonstrated *in vitro*, an altered methylation profile of *nucleosomal binding protein-1* promoter was found in genistein-treated neonatal CD-1 mice as compared to genistein untreated controls [86]. Another study demonstrated that genistein reactivates ERα expression in ERα-negative breast tumors, of importance in utilization of the available anti-estrogen treatments, *in vivo* in breast xenograft and spontaneous breast tumor mouse models [87]. Based on their findings, the authors further demonstrated that the ERα reactivation effect was enhanced synergistically when combined with a HDAC inhibitor in ERα-negative MDA-MB-231 breast cancer cells. In contrast, some *in vivo* studies have shown that genistein induced hypermethylation of cancer-related genes [88, 89]. These results correlate with a recent human trial, in which 34 healthy premenopausal women fed isoflavones, including genistein, daily through one menstrual cycle, showed hypermethylation in some of the key cancer-related genes [90].

4.5. Resveratrol

Resveratrol is abundant in grape skin, and also found in berries and peanuts, *etc.*. Resveratrol has been shown to possess potent anti-cancer properties. It acts on cancer cells by regulating the pathways of cell division and cell growth, apoptosis, inflammation, angiogenesis and metastasis, *etc.*. It has been shown to inhibit the growth of a wide variety of human cancer cells, such as breast, skin, lung, prostate and colon cancers [91–94]. Resveratrol has been shown to have a moderate inhibitory effect on DNMTs [80, 95]. Resveratrol treatment led to decreased DNMT1 and 3b expression *in vitro.* In a rodent model of estrogen-dependent mammary carcinoma, resveratrol treatment decreased DNMT3b expression in tumor samples but not in the normal tissues. miRNA expression was also found to be altered after resveratrol treatment in tumor *vs.* normal tissues *in vivo* [96]. Resveratrol treatment of human bladder cancer (EJ) cells was found to lead to remarkable S-phase arrest and apoptotic cell death accompanied by loss of phosphorylation of STAT-3, leading to downregulation of the STAT-3 pathway as well as decreased nuclear translocation of SIRT1 and p53 [97]. In another study, the chemopreventive effects of

resveratrol were demonstrated *in vivo* by pre-exposing aromatic hydrocarbon receptor (AhR) agonist-treated pregnant Sprague-Dawley rats with resveratrol, which is an AhR-antagonist, which led to lesser CpG methylation of the *BRCA-1* gene [95]. Collectively, these studies indicate that resveratrol possesses anti-cancerous activities and that these are mediated through multiple genetic and epigenetic modes of actions.

4.6. Other dietary phytochemicals

In addition to the various dietary phytochemicals described above, some other dietary phytochemicals have shown epigenetic modulatory activities in various cancers. For example, allyl derivatives from garlic such as allyl mercaptan, diallyl disulfide, S-allylcysteine, S-allylmercaptocysteine and allicin inhibit HDAC activities in human cancer cells [98–102]. Nian *et al.* [103] demonstrated competitive enzyme kinetics using a purified human HDAC8 enzyme and found that among the organosulfur compounds examined, allyl mercaptan was the most potent HDAC inhibitor. Further, the authors demonstrated that treatment of allyl mercaptan enhanced sp3 binding on the $p21^{CIP1/WAF1}$ promoter, which results in $p21^{CIP1/WAF1}$-mediated cell cycle arrest of cancer cells [103]. Grape seed proanthocyanidins (GSPs) are also bioactive phytochemicals that have been shown to have anti-cancer activities both *in vitro* and in *in vivo* models [104–106]. GSPs consist mostly of proanthocyanidins (>89%) with dimers, trimers, tetramers, and oligomers of monomeric catechins [105]. Treatment of A431 and SCC13 skin cancer cells with GSPs revealed inhibition of DNA methylation and histone modification leading to the activation of tumor suppressor genes [107].

In addition to the above described epigenetic modulatory phytochemicals, there are several other compounds for which some evidence of epigenetic modulation in cancer prevention and therapy is available but require more elaborative studies to pin-point their exact epigenetic modes of action [10, 108–110]. Examples of these phytochemicals include are apigenin, caffeic acid, anacardic acid and lycopene, however, all these dietary bioactive compounds induce epigenetic modulations to variable extents and with different patterns, indicating their potential in cancer chemoprevention. In Figure 2 we summarize the epigenetic modulatory capabilities of the major bioactive dietary phytochemicals.

5. Advantages and present challenges to epigenetic cancer chemoprevention: future perspectives

Epigenetic therapy has emerged as a gleaming beam of hope in the field of cancer therapeutics. Epigenetic alterations are the early events during carcinogenesis, therefore understanding the course and nature of these alterations may help the researchers to set biomarker profiles for different cancers. Dietary phytochemicals have shown potential of modulating all the major epigenetic pathways such as DNA methylation, histone modifications and miRNAs. These epigenetic alterations culminate into the alterations in the activity of cellular regulatory and metabolic pathways leading to loss of carcinogenicity of transformed cells. Natural dietary phytochemicals cause lesser cytotoxicity to the normal cells and they are widely available. The dietary epigenetically active compounds have added advantage of cost-effectiveness, oral bioavailability and wide range of gene targets. In

addition, they are also capable of functioning as sensitizers for chemotherapeutic drugs in the drug-resistant cancer types. The combination of chemotherapy and epigenetic therapy has shown promising results against multiple cancers.

In spite of several preclinical and clinical evidences supporting the potentiality of epigenetic therapy, there are multiple challenges which remain to be solved. First, the dietary compounds function *via* multiple mechanisms. This lack of specificity places a major hindrance in the druggability of these compounds. Second, since the epigenetic processes are reversible, unnecessary reversal of dietary compound-mediated epigenetic modifications might prevent effective cancer therapy. Third, although the epigenetically active compounds have shown better promises in chemoprevention, chemo-sensitization and maintenance therapy, their efficiency in monotherapy is compromised and variable among different cancers. Therefore, these compounds may not be a good choice for the first-line cancer therapy. Fourth, the early nature of epigenetic alterations requires early diagnosis of the disease to be treated by epigenetic therapy. Use of epigenetic therapy at later stages is not as effective due to accumulation of multiple genomic alterations in the tumor cells. A better understanding of the global patterns of epigenetic modifications induced by dietary compounds can establish improved insights into the chemopreventive strategies and the potential of dietary phytochemicals to inhibit carcinogenesis. Development of early diagnostic techniques might also help in the prevention and therapy of cancer by epigenetically active dietary compounds/phytochemicals.

6. Conclusion

Dietary phytochemicals are of particular interest in the field of cancer prevention and therapy. Many of these phytochemicals establish their anti-cancer activities through multiple pathways and mechanisms. Currently, there is a greater focus on their epigenetic modulatory and gene regulatory activities due to the transgenerational nature and reversibility of these mechanisms of action. These characteristics, as well as their generally low toxicity, position these bioactive natural compounds as crucial cancer chemopreventatives. This review article provides a brief insight into the mechanisms of action of some selected dietary phytochemicals and their epigenetic targets, including the inhibition of DNMTs and HDACs during the course of cancer prevention and therapy. Since cancer is a multi-stage process, it requires multi-targeted approaches for the development of an effective treatment regimen. Many of the bioactive phytochemicals possess more than one epigenetic target, and are thus capable of concomitantly upregulating tumor suppressor genes, downregulating tumor promoters and/or downregulating oncogenes in cancer. Further, exploration of combinations of bioactive phytochemicals having two different epigenetic modulatory capacities or targets is needed as such strategies could represent a major advance in the development of effective preventive and therapeutic approaches against cancer. The current body of research warrants further clinical studies that are needed to standardize the doses, routes of administration, organ specificity and bioavailability in humans. The current investigations also indicate that the use of these bioactive natural compounds regularly in the diet can be used as a preventive as well as therapeutic strategy for cancers of different organs/origins.

Acknowledgments

Abbreviations used

DNMT	DNA methyltransferase
MBD	methyl binding proteins
HDAC	histone deacetylase
HAT	histone acetyltransferase
EGCG	(−)-epigallocatechin-3-gallate

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Highlights

- Epigenetic changes in genome make significant effects on initiation of many diseases including the risk of cancers.

- Regular intake of some dietary phytochemicals appears to be the best option for the chemoprevention of cancers.

- Dietary components or phytochemicals have the ability to act as inhibitors of DNA methyltransferases and histone deacetylases.

- Dietary phytochemicals or ingredients can be combined with the therapeutic drugs for better treatment options.

- Dietary phytochemicals show the abilities to reverse epigenetic changes responsible for the risk of cancers of many organs, and reduce the toxicities of the therapeutic drugs.
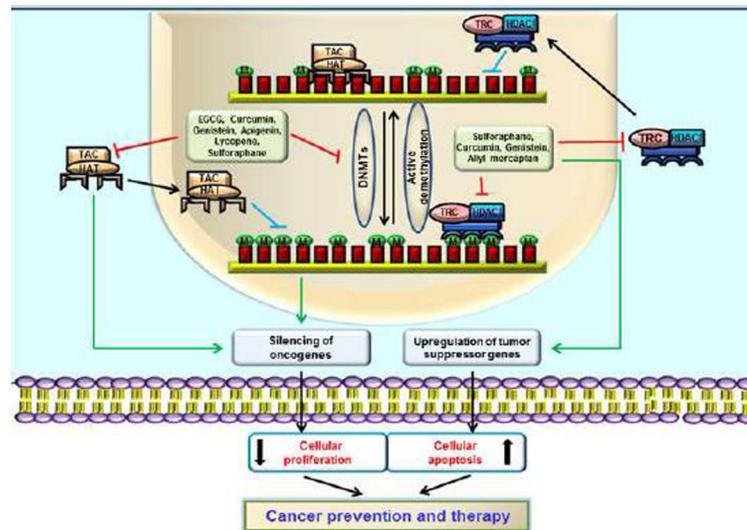
Figure 1.
Effects of bioactive phytochemicals on promoter methylation and gene regulations. Dietary phytochemicals inhibit DNMTs and induce active demethylation at CpG-rich gene promoters. The transcription activation complex (TAC), which contains HATs, in general, binds to the unmethylated gene promoter leads to silencing of oncogenes and upregulation of tumor suppressor genes. When the gene promoter is methylated by DNMTs, the TAC becomes unable to bind to the gene promoter and initiation of transcription is inhibited. The transcription repression complex (TRC) containing HDACs is recruited to the methylated DNA which in general, leads to silencing of the tumor suppressor genes. Further, the bioactive dietary supplements also directly destabilize different epigenetic remodelling complexes such as TAC and TRC to prevent their binding with respective promoter regions for gene activation/ repression. The dietary phytochemicals-mediated upregulation of tumor suppressor genes and down regulations of oncogenes are the central importance of cancer prevention and therapy. M, methyl group.
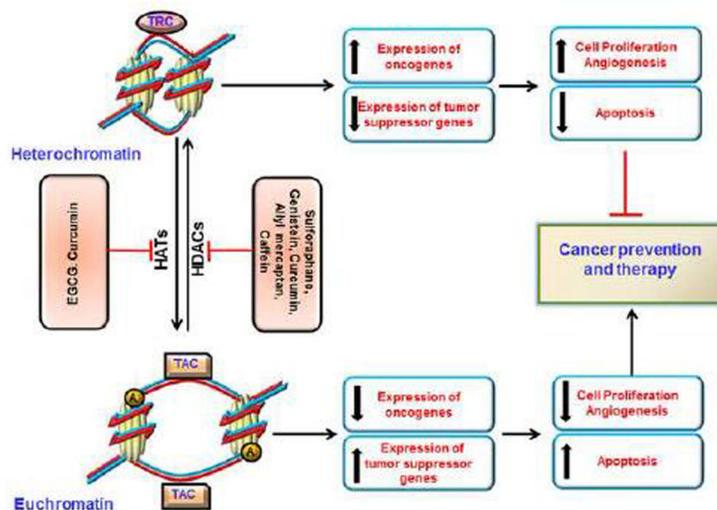
Figure 2.
Effect of bioactive dietary HAT and HDAC inhibitors on histone acetylation. HATs acetylate the lysine residues present in the histone tails thereby leading to formation of euchromatin state. This conformational change results in the binding of transcriptional activator complexes. The HDACs, in addition to deacetylation of these lysine residues, recruit transcriptional repressor complexes to the heterochromatin leading to gene silencing. The dietary HDAC inhibitors induce the expression of tumor suppressor genes and down regulation of oncogenes leading into suppression of carcinogenesis. A, acetyl group.