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Phytochemicals in cancer prevention: modulating epigenetic alterations of DNA methylation

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Abstract Cancer can take many years to develop from initiation to progression. The long period of development might represent an opportunity to use multi-functional, multi-targeted preventive drugs to block or reverse tumorigenesis. One path to cancer prevention could be to target and reverse the early epigenetic alterations. Unlike genetic mutations, they are potentially reversible and can be restored to their normal state. Epidemiological studies have revealed the close link between rich diets in bioactive compounds and the low incidence of different types of cancer. Thus, the study regarding the impact of bioactive nutrients on the epigenome has become widespread, with focus on the modulation of epigenetic mechanisms of gene expression, such as genomic DNA methylation. Following altered activity and expression of DNA methyl transferases and ten-eleven translocation enzymes, different types of cancers exert local DNA hypermethylation of gene promoters of tumor suppressor genes or of non-coding RNAs (microRNAs and long-noncoding RNAs), as well as global hypomethylation. Recently, the potential of phytochemicals to modulate epigenetic events in human health has become evident, although specific molecular mechanisms are still unclear. Phytochemicals and other bioactive dietary compounds can restore global and gene-specific promoter DNA methylation patterns by reactivating DNA methyltransferases or by providing the provision of methyl groups. Several natural products, such as EGCG, curcumin, sulforaphane, have shown DNMT inhibitory activity, but this property needs more in-depth investigations. This review focuses on the impact of modified DNA methylation pattern on early carcinogenesis and summarizes the effects/mechanism of phytochemical interventions on this type of epigenetic alterations.

Keywords Bioactive compounds · Chemoprevention · DNA hypermethylation · Epigenome · Sulforaphane

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Abbreviations

5-hmC	5-Hydroxymethylcytosine
5-mC	5-Methylcytosine
APC	Adenomatous polyposis coli
Bax	BCL2 associated X
BRCA1	Breast cancer 1
CCND2	G1/S-specific cyclin-D2
CDKN2A	Cyclin-dependent kinase inhibitor 2A
COMT	Catechol-*O*-methyltransferase
CYP	Cytochromes P450
DACT2	Dishevelled binding antagonist of beta catenin 2
DAPK	Death-associated protein kinase 1
DKK1	Dickkopf-related protein 1
DNMTs	DNA methyltransferases
EGCG	Epigallocatechin-3-gallate
EZH2	Enhancer of zeste homolog 2
GSTP1	Glutathione S-transferase P1
H3K27	Histone 3 lysine 27
H4K16ac	H4 acetylated at lysine 16
HATs	Histone acetyltransferases
HDAC	Histone deacetylase IDH2: isocitrate dehydrogenase 2
KDM4C	Lysine-specific demethylase 4C
LINE	Long interspersed nuclear element
LKB1	Liver kinase B1/serine/threonine kinase 11 (STK11)
MBD	Methyl-CpG-binding proteins
MeCP2	Methyl-CpG binding protein 2
MGMT	O6-methylguanine-DNA-methyltransferase
MLH1	MutL alpha1
MSH2	MutS protein homolog 2
NQO1	NAD(P)H: quinone oxidoreductase 1
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
PRC2	Polycomb repressive complex 2
RASSF1	Ras association domain-containing protein 1
RB1	Retinoblastoma1
SAH	S-adenosylhomocysteine
SAM	S-Adenosyl-methionine
SNF	Sulforaphane
SWI/SNF	Switch/sucrose nonfermenting (chromatin remodeling complexes)
TDG	Thymine-DNA glycosylase
TET	Ten-eleven translocation enzymes
TMS1	Target of methylation-induced silencing
TP73	Tumor protein p73

Introduction

Cancer is a deadly disease, affecting human health worldwide and causing a huge impact on economy and society. According to the latest World Health Organization (WHO) global report, cancer is responsible for an estimated 9.6 million deaths in 2018, and expected to increase with 20 million new cases by 2025. Around one-third of these deaths are caused by the five leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol consumption (https://www.who.int).

The development of cancer is a complex, multifactorial process characterized mainly by genetic mutations and epigenetic alterations. Interestingly, only 5–10% of all cancers are caused by inherited genetic mutations, whereas most cancers are triggered by environmental and lifestyle factors that can induce epigenetic changes in normal cellular development and function (Anand et al. 2008).

Cancer is a disease that can take many years to develop, from initiation to progression. For example, all the common epithelial cancers (lung, colorectal, breast, prostate, pancreas and ovary) have a long latency period, often 20 years or more. By the time they are clinically detectable, the cells may harbor hundreds of mutations in different genes (Sporn 2011). The long-term development of certain types of cancer could represent a major opportunity to use multifunctional, multi-targeted preventive drugs in order to block or reverse cancer-related modified cells. One path for cancer prevention could be to target and reverse the early epigenetic alterations that, unlike genetic mutations, are potentially reversible and can be restored to their normal state.

The epigenetic mechanisms are regulating gene expressions through genomic DNA methylation, histone post translational modifications, chromatin remodeling, and expression of non-coding RNAs (microRNAs and long non-coding RNAs). Each epigenetic mechanism is controlled by specific protein classes which attach, remove, or maintain specific chemical groups that constitute epigenetic marks for activation or inactivation of the gene transcription. The link between epigenome (epigenetic regulatory proteins and chemical marks), epigenetic mechanisms and gene expression form a complicated "feedback"

network that regulates and organizes cellular functioning at the molecular level (Dawson and Kouzarides 2012). When this regulatory circuit is discontinued by internal or external factors, normal physiological functions are affected, leading to tumor initiation process (Timp and Feinberg 2013).

Recent advances made in epigenetic field and cancer research showed that genetic and epigenetic mechanisms are not separate events in cancer; they interconnect and influence each other during tumorigenesis (You and Jones 2012). Alterations in epigenetic mechanisms can lead to genetic mutations; genetic mutations in epigenetic regulators lead to an altered epigenome (Timp and Feinberg 2013). Furthermore, evidence have suggested that epigenetic modifications might occur early in tumorigenesis and some of them even precede genetic mutations during cancer initiation (Feinberg et al. 2006). The abnormal proliferation of cells, due to accumulation of genetic and epigenetic aberrations, causes deregulation of major cellular processes, including cell cycling, DNA damage response, differentiation, and apoptosis.

Epidemiological studies (Zamora-Ros et al. 2014; Edmands et al. 2015; Sun et al. 2017) revealed that there is a close link between rich diets in bioactive compounds and the low incidence of different types of cancer. Over the recent years, studying the effects of bioactive nutrient treatment on the epigenome has become widespread, and it is currently certain that they can modulate epigenetic mechanisms of gene expression, such as genomic DNA methylation, acetylation or methylation of lysine residues from histones H3 and H4. Changes in DNA methylation have been recognized to be among the most common molecular alterations in human neoplasia and hypermethylation of gene-promoter regions is being revealed as one of the most frequent mechanisms of gene function loss.

This review focuses on the impact of modified DNA methylation pattern on early carcinogenesis and summarizes the effect/mechanisms of phytochemical interventions on this type of epigenetic alteration. The relationships between changes in DNA methylation pattern and lack of bioactive compounds intake, as well as the benefits of phytochemicals as prevention and/or early intervention in cancer, are also discussed.

DNA methylation as epigenetic regulator of gene expression

DNA methylation is the major epigenetic mechanism that provides a stable and reversible mechanism for gene silencing; it plays an important role in regulating gene expression, chromatin architecture and chromosome stability.

DNA methylation at the 5 position of cytosine (5-mC) is a key epigenetic mark that is critical for various biological and pathological processes. It consists in the addition of a methyl group from the universal methyl donor, S-Adenosyl-methionine (SAM), to the cytosine at the CpG dinucleotide residues. The members of the DNA methyltransferase (DNMT) family directly catalyze the addition of methyl groups onto DNA and are the major players as epigenetic modifiers. DNMT3A and DNMT3B are de novo methyltransferases by initiating DNA methylation and establishing the methylation patterns independently of replication. DNMT1 maintains the original pattern of DNA methylation in a cell lineage manner, and has the ability to repair DNA methylation (Mortusewicz et al. 2005). The maintenance mechanism mediated by DNMT1 is crucial to ensure the faithful reestablishment of 5-mC on the newly synthesized strand after DNA replication (Mortusewicz et al. 2005). Moreover, there is an active cooperation between all three enzymes in order to maintain DNA methylation at densely methylated regions, repetitive elements, and imprinted genes (Liang et al. 2002).

The CpG dinucleotides, known as CpG islands, are preferentially located in the proximal promoter end of approximately 60% of genes in the human genome. Unmethylated CpG islands correspond to either active transcription or a poised state, where genes can be expressed if the appropriate molecular signals are present (Suzuki and Bird 2008). In addition, large methylated domains are found predominantly in the long interspersed and tandem repetitive sequences that represent approximately 70–90% of the CpG dinucleotides in the entire genome (Rollins et al. 2006). These methylation patterns of the genome are vital for both chromosomal and genomic stability, possibly through the repression of retroviral transposons (Jones 2012). Consequently, the epigenetic mechanism through DNA methylation facilitates the organization of the genome into active (euchromatin) and inactive

regions (heterochromatin) with respect to gene transcription.

DNA methylation is essential for normal mammalian development, function and differentiation by its epigenetic control of protein-coding RNAs or non-coding RNAs expression.

The 5-mC epigenetic mark is chemically stable and its presence at the promoter sites induces transcriptional inhibition by sterically blocking the further binding of transcription factors (Deaton and Bird 2011). Sequentially, the MBD proteins are recruited at methylated DNA and further facilitate the formation of compact, inactive heterochromatin. The unmethylated CpG sites bind switch/sucrose nonfermenting (SWI/SNF) chromatin remodeling complexes, histone acetyltransferases (HATs) and histone methyltransferases (HMTs), which label the chromatin with histone transcriptionally active marks (Bannister and Kouzarides 2011). The sequential recruitment of different protein complexes, followed by histone acetylation, demonstrated that the formation of open transcriptionally active chromatin is a dynamic process where layers of epigenetic regulators participate to the gene expression mechanism (Memedula and Belmont 2003; Bintu et al. 2016).

For decades, DNA methylation has been considered to be a non-reversible reaction, until the discovery of 5 hydroxymethylcytosine (5-hmC) and the TET family of enzymes.

The active demethylation consists of a series of successive oxidation reactions catalyzed by TETs. First, 5-mC is converted to 5-hmC; afterwards, the methyl group is removed by a TDG-mediated base excision mechanism (Oswald et al. 2000; Weaver et al. 2004; Wu and Zhang 2017).

The 5-hmC epigenetic mark is also associated with differentiation and normal development, (Ficz et al. 2011) and the levels and distribution of 5-hmC might vary in different tissues, with the highest accumulation being found in the brain (Chen and Riggs 2011; Wu and Zhang 2017). While 5-mC is associated with closed heterochromatin, 5-hmC is associated with DNA demethylation and an open active chromatin state.

The role of 5-hmC in gene expression regulation has still not been fully elucidated. The unique genomic distribution patterns of TET1 and 5-hmC at the transcription starting sites and promoters, as well as gene bodies, are suggesting that they might regulate gene expression through modulating chromatin accessibility or by inhibiting repressor binding (Williams et al. 2011; Wu and Zhang. 2017). As an epigenetic reader protein, methyl-CpG binding protein 2 (MeCP2) has similar affinity to both DNA epigenetic marks (Mellén et al. 2012), but it has been observed that the 5-hmC level is negatively correlated with MeCP2 abundance. One explanation could be that the binding of MeCP2 to 5-mC can possibly hinder the production of 5-hmC (Mellén et al. 2012). Therefore, 5-hmC and MeCP2 might constitute a cell-specific epigenetic mechanism for the regulation of gene expression and remodeling the chromatin structure. The interconnection between DNA methylation and the demethylation processes is exemplified in Fig. 1, where the enzymatic processes catalyzed by the epigenetic regulator proteins (DNMTs, TETs) are changing other epigenome marks (5-mC, 5-hmC).

DNA methylation is a reversible, enzymatically controlled mechanism of gene expression, involved, among other processes, in normal embryogenesis, tissue differentiation and chromosome stability. Modifications in any of the layers controlling this epigenetic process can lead to carcinogenesis, as discussed in the following section.

Epigenetic alterations in carcinogenesis: DNA methylation

Cancer methylation has been characterized by global hypomethylation together with local, de novo promoter CpG islands hypermethylation (Jones and Baylin 2007; Sharma et al. 2010), including those of classic tumor suppressors (Shen and Laird 2013). Furthermore, some local variations in methylation pattern at only several key genome loci are sufficient for cancer initiation (Plass et al. 2013). Importantly, the altered patterns of DNA epigenetic marks (5-mC, 5-hmC) are frequently accompanied by a critical imbalance in transcriptional programs involving differentiation and stem cell maintenance, thereby could participate to tumor initiation and sustaining cancer cells growth (Jones and Baylin 2007).

Actually, a series of studies showed that the DNA methylation is a driver of tumorigenesis and that cancer cells suffer additional epigenetic alterations which are essential for cancer cell survival (Baylin and Herman 2000; De Carvalho et al. 2012). Furthermore,

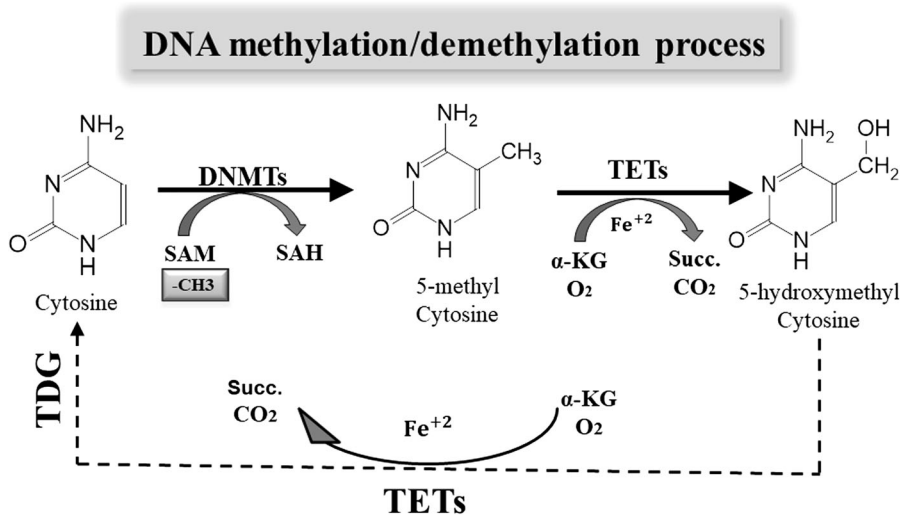


Fig. 1 DNA methylation and demethylation process. DNA methylation occurs at the 5′ position of cytosine, within CpG dinucleotides. DNMTs catalyze the transfer of the methyl group to cytosine and generate 5-mC using SAM as methyl donor and producing SAH. DNA demethylation is a multi-step oxidation process catalyzed by TETs methylcytosine dioxygenases family that uses Fe^{2+} and α—ketoglutarate as cofactors or substrates, and generates succinate and CO_2. In the first step of demethylation process, the 5-mC is converted to 5-hmC, and after several oxidation reactions the methyl group can be remove by TDG-mediated base excision repair mechanism

the fact that DNA hypermethylation could be an early event in carcinogenesis is supported by the finding that adjacent normal tissues also harbor altered DNA methylation patterns (Taby and Issa 2010). Only ∼ 15% of the genes methylated in cancer samples were actively transcribed in normal tissue; moreover, they were already inactivated by methylation in precancerous tissue (Keshet et al. 2006).

Local DNA hypermethylation

The list of aberrantly methylated genes in cancer is steadily growing, including those with major impact on cellular pathways involved in carcinogenesis: detoxification (GSTP1), DNA repair (MGMT, BRCA1, MLH1), cell cycle (CDKN2A/p16-INK4, CCND2, RB1), Ras signaling (RASSF1), Wnt signaling (APC, DKK1), apoptosis (DAPK, TMS1, TP73) and so on. Most of these genes are found to be inactivated in many types of cancer and the DNA methylation of promoter's gene could be considered as early event in tumor initiation, which could represent the major target for cancer prevention.

For example, the hypermethylation and silencing of the intracellular detoxification enzyme GSTP1 is considered a molecular hallmark of prostate cancer and was already implemented in clinical diagnosis. The silenced GSTP1 was found at the earliest stages of prostate cancer initiation and was observed in more than ∼ 90% of the tumors, and in several other types of cancer (Kim et al. 2011; Witte et al. 2014). The loss of GSTP1 enzymatic detoxification activity may explain the well-known sensitivity of human prostatic carcinogenesis to environmental factors and the demethylation of GSTP1 promoter could become a target for epigenetic chemoprevention (Jerónimo et al. 2011).

Similarly, methylation mediated silencing was reported in other important DNA repair genes, such as MGMT, BRCA1, BRCA2 and MSH2 at a pan-cancer level, and are frequently observed in multiple cancer types, for instance lung, gastric, colorectal, leukemia, brain, liver, breast, and prostate (Witte et al. 2014). The inactivation of genes in DNA repair pathways will further propagate the carcinogenic state by allowing cells to accumulate additional genetic lesions. MGMT, which normally protects from mutations occurring at guanine bases, is silenced by hypermethylation events and often occurs early in tumorigenesis (Witte et al. 2014). The inactivation of

MGMT is predominantly epigenetic, for example its promoter is methylated in 44% of human esophageal squamous cell carcinomas, while appearing mutated in only 17.5% of the patients (Keshet et al. 2006; Weisenberger 2014). These results suggest that a primary epigenetic defect in mismatch repair mechanisms can accelerate the rate of accumulation for additional mutations in cancer cells.

In addition, the silencing of transcription factors could indirectly silence or downregulate a large number of other genes. For example, silencing a key tumor suppressor p16 (encoded by the CDKN2A gene in humans) is related with aberrant promoter hypermethylation and it is a common epigenetic mark in human cancers. Mice bearing a hypermethylated p16 promoter had a higher incidence of developing spontaneous cancer during ageing. But when the mice carried inactivating germline mutations in one allele of p16 and epigenetic alterations in the other allele, they showed early onset of tumors and shorter survival time (Yu et al. 2014). Put together, these data demonstrate that epigenetic mutations are able to act as driver events in tumor initiation and progression.

Interestingly, dysregulation of DNA methylation status at promoters of non-coding RNAs (miRNAs and lncRNAs) could also promote carcinogenesis (Kozaki and Inazawa 2012; Pop et al. 2018). Aberrant microRNA expression in cancer has been associated with epigenetic regulation, such as DNA methylation and histone modifications. It is estimated that the transcription of 10% of all microRNA species is controlled by DNA methylation (Kozaki and Inazawa 2012) and approximately 50–70% of microRNA genes are located at fragile genomic sites that are frequently affected during carcinogenesis (Starczynowski et al. 2011). Also, microRNAs control and regulate expression of major epigenetic modifier proteins involved in DNA methylation processes, including DNMTs and TETs. Several studies have demonstrated that alterations in the expression of miRNAs are prominent events during the early stages of liver carcinogenesis and may predict susceptibility to cancer development (Anwar and Lehmann 2014). In vivo studies showed that a methyl-deficient diet induced hepatocellular carcinogenesis associated with global DNA hypomethylation, and with changes in several miRNA expression, which could be reversed by restoring dietary methyl donors (Parasramka et al. 2012).

Global DNA hypomethylation

The global lower methylation level (hypomethylation) is a common epigenetic alteration in cancer, especially at Long Interspersed Nuclear Element (LINEs) regions, which are a group of retrotransposons widespread in human genome. They are translated into proteins that act as reverse transcriptase able to reproduce DNA copies, which are then relocated into new genomic sites. In human somatic cells, the LINE-1 is heavily methylated and thus is mostly suppressed, maintaining the genomic stability by avoiding retrotransposition to other genomic loci (Lee et al. 2012). Several studies showed that LINE-1 is gradually hypomethylated during cancer progression, with the first signs of global methylation changes initiated early in carcinogenesis (Slotkin and Martienssen 2007; Kitkumthorn and Mutirangura 2011). Hypomethylation of LINE-1 triggers the active process of genome reorganization, and the relocations of these interspersed repetitive sequences are a source of endogenous mutagenesis and polymorphism in the premalignant and malignant cells (Kitkumthorn and Mutirangura 2011; Lee et al. 2012).

Also, the loss of 5-hmC is an epigenetic hallmark of aggressive tumors, such as melanoma, glioblastoma or ovarian cancer, with both diagnostic and prognostic implications (Tucker et al. 2018). The overexpression of active proteins IDH2 or TET2 in animal models for human melanoma was an efficient way to increase the 5-hmC level (Lian et al. 2012). Likewise, pretreatment with DNMTs inhibitors restored the 5-hmC patterns via enhanced levels of TET family enzymes, both in vitro and in vivo experiments (Tucker et al. 2018).

Other epigenetic alterations

There are approximately 40 epigenetic regulators that exhibit some form of alteration in cancer (Jones and Baylin 2007). The most prominent are somatic mutations in the proteins involved in DNA methylation and demethylation mechanisms. For example, somatic heterozygous mutations in DNMT3A are found in $\sim$ 20% of patients with acute myeloid leukemia (AML) and recent evidence suggest that at least some of these mutations exhibit dominant-negative effects by inhibiting the function of the wild-type DNMT3A allele (Kim et al. 2013). The

epigenetic regulators expression could be directly altered in various cancers, but other mutated genes may impinge on the proper function of these enzymes. For example, recurrent mutations in the IDH1 and IDH2 alter their enzymatic activity and consequently the 2-hydroxyglutarate is produced, which may inhibit several dioxygenases, including TET2 and KDM4C (Feinberg et al. 2016). This indicates that not only the epigenetic factors are directly altered in tumorigenesis, but also that mutations in other pathways can have an impact on the regulation of gene expression. Genetic alterations in TET1 and TET2 have also been identified in leukemia and solid tumors (Wu and Zhang 2017), with direct effects on epigenetic degradation of 5-hmC and 5-mC patterns. Coordinated epigenome changes can also be achieved via the interaction of multiple epigenetic regulators, which guide different enzymatic activities to the same locus. Recent results have demonstrated that the PRC2 complexes are recruited to specific DNA regions based on DNA sequence and transcription factor occupancy. The protein EZH2, that mediates repressive chromatin formation through the deposition of H3K27 methylation, and DNMT enzymes, act at the same locus for the coordination of repressive histone and DNA methylation marks (Viré et al. 2006). Thus, the crosstalk between the different layers of the epigenetic mechanism could amplify early epigenetic changes, leading to the development of cancer.

The overexpression of DNMTs represents a common feature in a variety of tumors, and results in local DNA hypermethylation and oncogenic activation. Highly expressed DNMT3A and DNMT3B have been found in a large number of patient specimens, with increased DNMT3A expression in hepatocellular carcinogenesis, where intense hypomethylated genomic regions were also observed (Zhao et al. 2010). Moreover, high expression levels of DNMT3B have been correlated with the epigenetic inactivation of BRCA1 in sporadic breast tumors (Butcher and Rodenhiser 2007). As a result, the overexpression of DNMTs in many cancers promoted local and global DNA methylation aberrations related with genomic instability and oncogenic pathways activation.

In conclusion, there are several epigenetic mechanisms related to DNA methylation patterns, from local hypermethylation of specific gene promoters to global DNA hypomethylation, with impact on cellular processes which dysregulated can lead to carcinogenesis, as we exemplified in Fig. 2.

Whether dysregulation of DNMTs and TETs enzymes can be modulated by phytochemicals in an efficient manner, in order to counteract various mechanisms acting in tumor initiation and progression, will be the topic of the following section.

Phytochemicals in cancer prevention

Cancer chemoprevention implies the use of dietary or pharmacological compounds to prevent, inhibit, or even reverse the process of carcinogenesis before clinical manifestation of the disease. Therefore, effective chemoprevention requires the use of compounds that inhibit specific molecular steps in the carcinogenic pathway, including the epigenetic alterations that are early and potentially reversible events. Substantial experimental evidence and epidemiological studies indicate the potential importance of dietary phytochemicals and nutritional factors in cancer prevention. Diets rich in fruits and vegetables could prevent at least 20% of all cancers (www.who.int). In addition, a recent study showed that more than 49% of all 175 small molecules approved for cancer therapy were natural products or directly derived from them (Newman and Cragg 2016). Natural products with bioactive components have gained increasing attention in cancer prevention and therapy, due to their compatibility with biological target sites and less induced toxicity to normal cells (Remely et al. 2015). Several preclinical studies have reported that many phytochemicals with anti-inflammatory, anti-oxidation and anti-proliferative properties can prevent cancer initiation and development by inducing apoptosis and activating antioxidant enzymes (Venkatachalam et al. 2016; Leone et al. 2017). However, the translation of chemopreventive properties of phytochemicals to clinical practice has not been yet achieved.

Recently, the potential of phytochemicals to modulate epigenetic events in human health has become evident, although specific molecular mechanisms are still unclear. Among the impressive number of phytochemicals with anti-tumoral properties, some polyphenols and organosulfur compounds are part of the dynamic interaction between the genome and the environment with specificity at physiological

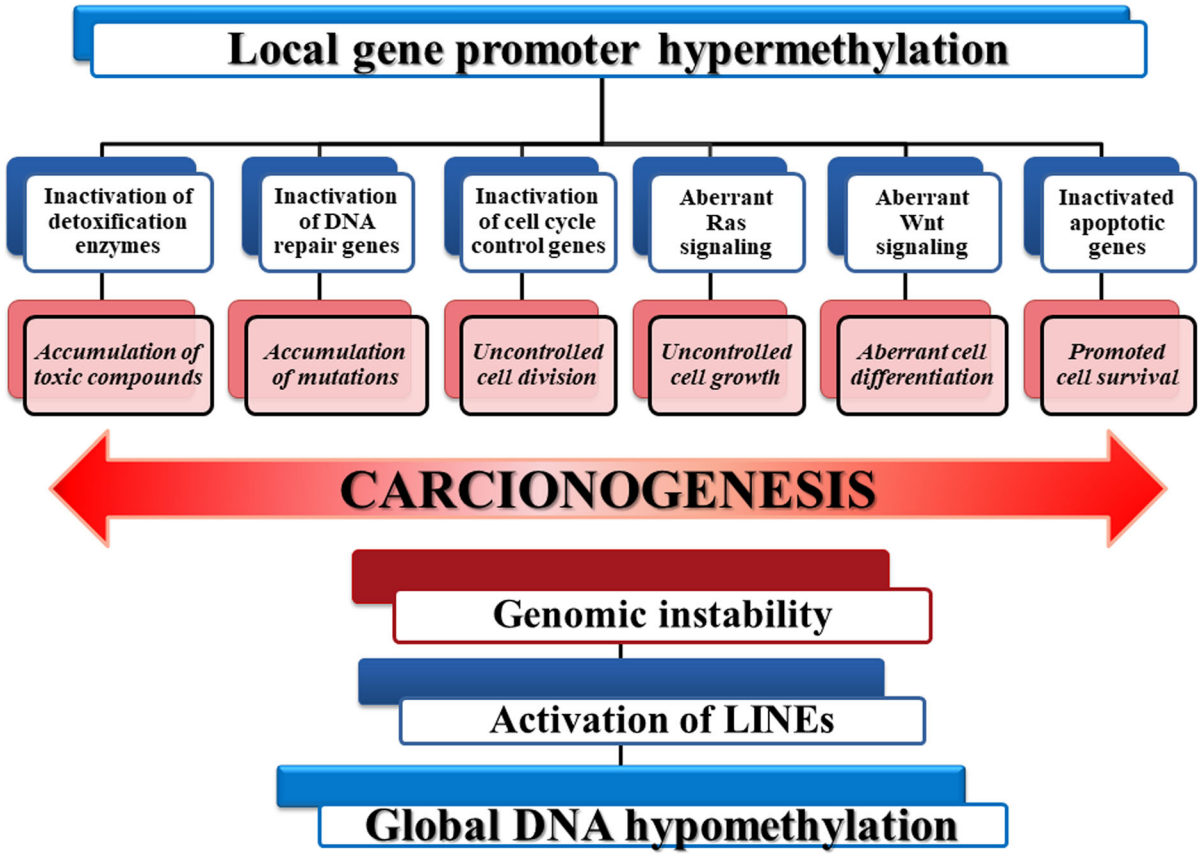


Fig. 2 Epigenetic alterations of DNA methylation in carcinogenesis. There are two main epigenetic changes of DNA methylation related to cancer initiation and development—global DNA hypomethylation and local gene promoters hypermethylation. Each leads to specific events, such as activation of LINEs or, inactivation of genes involved in specific cellular processes: detoxification, DNA repair genes, and tumor suppressor gene respectively. Isolated or in summation, these alterations can eventually lead to cancer

concentrations, and well known to modulate mechanisms underlying in human health.

DNA methylation dysregulations induced by poor nutrition

Recent studies have highlighted the cross-talk between cancer metabolism and the epigenome. Metabolites such as SAM, acetyl-coA, and AMP are required for epigenetic mechanisms such as DNA and histones methylation, histone acetylation or phosphorylation (Donohoe and Bultman 2012; Newman and Maddocks 2017). The metabolic pathways and enzymes that supply these key compounds are therefore critical for the maintenance and adaptation of the epigenome. Indeed, a diet with deficit in methionine decreases SAM levels, leading to diminished DNA and histone methylation with significant effects upon gene expression (Donohoe and Bultman 2012; Parasramka et al. 2012). The metabolism of folate, betaine, choline, and methionine are interrelated, and the deficiency of one nutrient can cause metabolic and functional disturbances. A diet poor in methyl donor contributors can have a rapid effect on global DNA methylation pattern. For example, within 1 week, the global DNA hypomethylation and increased levels of mRNA for oncogenes c-fos and c-myc were observed in liver tissues of Fischer rats fed with methionine and choline-deficient diet. After restoration of proper methyl donors rich diet, the global and local DNA methylation pattern returned to normal within 1–2 weeks (Niculescu and Zeisel 2002). In the case of longer exposure to methyl deficient nutrition (18–36 weeks), the epigenetic alterations of DNA

methylation could not be reversed by reintroducing the animal models to the right diet. The global DNA hypomethylation pattern and altered hepatic foci in their liver were irreversible (Pogribny et al. 2006). These data provide further experimental evidence to demonstrate that epigenetic alterations may contribute to the initiation and promotion of liver carcinogenesis.

During methionine starvation, the other metabolic pathways, such as serine cycle could provide cofactors to recycle homocysteine to methionine. Indeed, the serine-dependent de novo ATP synthesis might support the conversion of methionine to SAM. In this case the ATP pool is reduced and this can have a direct impact on the rate of SAM generation and methylation of DNA (Maddocks et al. 2016). Recently, the specific contribution of one-carbon metabolism-dependent DNA methylation in pancreatic cancer has been explored. The loss of the serine–threonine kinase, LKB1, promotes tumorigenesis in KRAS mutant pancreatic cancer, accompanied by increased levels of global DNA methylation and increased expression of DNA methyltransferases for which SAM is a critical cofactor. This serine-dependent DNA methylation upon the loss of LKB1 in KRAS-mutant cells contributes to tumor growth, presumably through the activation of several oncogenes (Kottakis et al. 2016). Dietary and genetic perturbation of metabolic pathways could lead to dysfunctional DNA synthesis and DNA methylation, connecting directly the cancer metabolism to the epigenetic mechanism.

The cellular DNA methylation processes involve a series of catalytic reactions, which result in the generation of the principal methyl donor, SAM, followed by methyl group transfer reactions. As a consequence of methyl group transfer, SAM is converted to SAH, which binds to methyltransferases and induces product inhibition (Mortusewicz et al. 2005). Therefore, maintaining the proper ratio of SAM to SAH is a determinant factor for DNA methylation mechanism, since this ratio dictates methyltransferase activity in vivo. Disturbance of this system may be caused by dietary imbalances and in consequence the major epigenetic regulatory enzymes are affected, dysregulating DNA methylation pattern (Stefanska et al. 2012). In a pre-malignancy pathological condition, the appropriate consumption of a diet rich in methyl donor nutrients may interfere with early carcinogenesis events leading to cancer prevention.

The dietary phytochemicals may exert their chemoprevention activities by indirectly modulating DNMTs activities through altering the SAM/SAH ratio and having effects upon interference with cellular metabolism. The flavanol-rich diets contain polyphenols with catechol structures that can be methylated by catechol-*O*-methyltransferase (COMT) enzyme using SAM as a methyl donor (Bistulfi et al. 2010; Chen et al. 2010). This methylation reaction results in the demethylation of SAM and formation of SAH, which is a potent and selective inhibitor of DNA methyltransferase (Zhu et al. 2010). Phytochemicals and other bioactive dietary compounds can restore global and gene-specific promoter DNA methylation patterns by reactivating DNA methyltransferases or providing the provision of methyl groups. Therefore, phytochemicals could epigenetically modulate gene expression by changing the chromosomal integrity and stability with benefits on health conditions.

Phytochemicals modulating the epigenetic alterations of DNA methylation pattern

Numerous studies have demonstrated that certain dietary phytochemicals inhibit tumor growth by affecting epigenetic signaling pathways both in vitro and in vivo (Remely et al. 2015).

The dietary phytochemicals with epigenetic modulation activities of DNA methylation levels can be categorized in three group based on their mechanism of action (Ho et al. 2011): (1) the phytochemicals which directly donate the methyl group and act as co-substrates in DNA methylation process; (2) the phytochemicals that indirectly modulate the DNMTs activity by affecting the methyl pool; and (3) the phytochemicals that act as direct DNMT enzyme inhibitors, which are amongst the most promising bioactive natural products candidates for cancer prevention and therapy.

Polyphenols are the largest class of plant secondary metabolites that are mainly found in fruits, vegetables, cereals, and beverages. Besides common antioxidant or anti-inflammatory activity, many polyphenols might modulate early epigenetic alterations related to cancer prevention. The polyphenol groups include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), lignans, stilbenes, and flavonoids (Hardman 2014).

Flavonoids are the most representative group of dietary polyphenols with diverse biological activities including anti-bacterial, anti-viral, analgesic, hepatoprotective, apoptotic, and estrogenic functions (Kumar and Pandey 2013). The chemoprevention activity of flavonoids might be mediated by certain epigenetic mechanisms, including modulation of DNA methylation status and histone methylation and acetylation (Jiang et al. 2015; Guo et al. 2018; Khan et al. 2018). The flavone apigenin can restore the silenced status of Nrf2 gene in skin epidermal cells by reducing the expression of three DNA methyl transferase proteins (DNMT1, DNMT3A, and DNMT3B) as well as the expression of some HDACs (Shukla and Gupta 2010). In addition, apigenin, together with another flavone, luteolin, has been reported to act synergistically to modulate the DNMT activity in esophageal squamous cell carcinoma line (Busch et al. 2015). Research suggests that a diet rich in flavones might decrease the risk of certain cancers, including breast, digestive tract, skin, and prostate cancer (Li and Tollefsbol 2010; Shukla and Gupta 2010).

Some flavonoids have a selective DNMTs inhibitory activity, for example kaempferol inhibits DNMT1 and DNMT3B but not DNMT3A′ enzymatic activity in bladder and CRC cancer (Banerji 2017; Lu et al. 2018).

Genistein is the most potent DNMT inhibitor amongst isoflavones, capable to reactivate methylation silenced genes such as RARb, p16INK4a, and MGMT in esophageal squamous carcinoma and prostate cancer cells (Fang et al. 2005; Dietz et al. 2016). Besides, genistein treatment of benign and tumor breast cells depletes human telomerase reverse transcriptase (hTERT) activity, the catalytic subunit of telomerase, which is overexpressed in 90% of cancers, through epigenetic modulation that involves decreasing the DNMTs expression levels and concomitant with hyper-methylation of H3 K9me3 and hypomethylation of H3K4me2 chromatin marks (Li et al. 2009).

The major catechin from green tea, EGCG, exerts its chemoprevention effect by blocking cell proliferation and transformation and promoting apoptosis and cell cycle arrest in several human cancer cell lines including leukemia, melanoma, breast cancer, lung, and colon (Singh et al. 2011; Schramm 2013). The molecular mechanism underlying EGCG chemoprevention action is related with the regulation of several signal transduction pathways including: MAPK, PI3K/AKT, Wnt, Notch, and NF-κB (Pandey et al. 2010; Moseley et al. 2013; Schramm 2013; Khan et al. 2018). Additionally, EGCG has been demonstrated to induce the increase of tumor suppressor expression, such as: p53, p21, p16 and Rb with certain roles in chemoprevention (Pandey et al. 2010; Du et al. 2012). Moreover, EGCG possesses a chemopreventive effect against a broad spectrum of carcinogens by inhibiting the chemical induced colon, liver and skin carcinogenesis in several animal models (Henning et al. 2013).

EGCG exerts its epigenetic modulator capacity of DNA methylation processes indirectly, by acting as a substrate for COMT catalyzed methylation reaction (Bistulfi et al. 2010) or directly by inhibiting DNMT1 and DNMT3A enzymatic activities through blocking their catalytic sites. Molecular docking studies indicate that the gallic acid moiety of EGCG can accommodate in the hydrophilic active pocket of DNMT1 (Lee et al. 2005). Fang et al. demonstrated that EGCG binds to DNMT and competitively inhibits the enzymatic activity yielding to the reactivation of methylation-silenced genes in prostate cancer cells (Fang et al. 2003). In addition, treatments of different PCa cell lines with EGCG have determined a dose- and time-dependent re-expression of GSTP1 enzyme concomitantly with the down-regulation of DNMT1 (Naponelli et al. 2017). Recent studies have demonstrated that EGCG induces epigenetic changes modulating hTERT activity through inhibition of DNMT and HAT activities (Li and Tollefsbol 2010; Du et al. 2012). All these data support the idea of EGCG as a key active nutrient for cancer inhibition through epigenetic control; however polyphenolic catechins generally exhibit poor oral bioavailability. Further investigation is required to improve EGCG absorption and metabolic biotransformation, in order to increase its potential effect in cancer prevention and therapy through epigenetic modulation.

Curcumin (diferuloylmethane) is a polyphenolic compound derived from turmeric (Curcuma longa Linn) with remarkable medicinal properties, mainly with anti-inflammatory and anti-cancer effects. Curcumin has been shown to modulate multiple intracellular pathways associated with proliferation, survival, invasion, apoptosis, and inflammation (Park et al. 2013; Jiang et al. 2015, Guo et al. 2018). In silico molecular docking studies revealed that curcumin can

block or inhibit the catalytic site of DNMT1, thus resulting in decreased enzymatic activity. In vitro experimental studies validated the DNMT1 and DNMT3B inhibition activity of curcumin in several human cancer cell lines (Jiang et al. 2015; Guo et al. 2018). Similarly, in vitro and in vivo experiments showed that curcumin and its synthetic analogue (FN1) were able to restore the activity of Nrf2 gene by hypomethylation of its promoter and through inhibition of DNMTs activity, hence activating anti-oxidant pathways (Li et al. 2016a, b).

Several examples of phytochemicals involved in epigenetic modulation of DNA methylation dysregulation and chemoprevention in different carcinogenesis are presented in Table 1, such as well-known resveratrol, quercetin and others.

We will exemplify next with one phytochemical which exerts its epigenetic modulator capacity on various types of epigenetic alterations in carcinogenesis and could interfere with different layers of epigenetic mechanism, sulforaphane (SNF).

Sulforaphane belongs to Brassicaceae family and represent the most effective chemopreventive agent among isothiocyanate (ITC) group of organosulfur compounds. Many studies have shown that SFN is an effective chemopreventive agent that has anti-proliferative, anti-inflammatory, anti-angiogenic, and anti-oxidative effects, as well as induction of differentiation, apoptosis, and cell cycle arrest in several types of cancers (Cao et al. 2018). SFN induces its chemopreventive effects partly by activation of phase I CYP enzymes and phase II detoxification enzymes, leading to restored mitochondrial function and reduced lipid peroxidation (Kwon et al. 2007). In human breast, colon and hepatocellular carcinoma, the chemopreventive activities of SFN are mediated, at least in part, through Nrf2 pathway activation, which modulates phase 2 detoxification enzymes, including NAD(P)H: quinone oxidoreductase 1 (NQO1) and GST (Cao et al. 2018). A recent study demonstrates that SFN can activate the Nrf2 pathway in breast cancer cells, acting as an epigenetic modifier to regulate COMT expression to influence estrogen metabolism (Cao et al. 2018).

Numerous in vivo studies on murine models of colon, prostate, oral and pancreatic cancer showed the chemopreventive role of SNF by inhibiting tumor growth (Hsu et al. 2011). Interestingly, topical application of SFN for a long period of time inhibited chemical induced skin carcinogenesis in C57BL/6 mice, whilst no such chemopreventive effects of SNF were elicited in the Nrf2-deficient mice (Kwon et al. 2007).

There has been an increased interest in SFN recently, due to its potency to influence epigenetic processes through targeting key epigenetic modulators such as DNA methyltransferases and HDACs, which may lead to local or global alterations of epigenetic hallmarks resulting in subsequent gene transcription and expression level changes (Khan et al. 2018). Also, SFN modulates DNA demethylation by downregulation of the expression of DNMT1 and DNMT3B, subsequently leading to induced demethylation of cyclin D2 gene promoter and expression in cancer cells (Hsu et al. 2011). Similarly, in prostate cancer cells, SNF has been reported to be able to restore the expression of silenced GSTP1 by a mechanism involving promoter demethylation and increased histone acetylation. These effects are associated with increased expression of the CDKNs p21 and p27, which are negative cell cycle regulators (Hsu et al. 2011). Moreover, the SNF inhibition of the growth of prostate cancer PC-3 tumor xenografts could be correlated with inhibited HDAC activity. In human subjects, a single dose of 68 g broccoli sprouts decreased HDACs activity significantly in peripheral blood mononuclear cells (PBMC) at 3 and 6 h following consumption (Myzak et al. 2007).

Recently, a comprehensive study of SNF chemopreventive effect on three breast cancer showed that SFN provoked cell cycle arrest and senescence are mediated by epigenetic changes, namely global DNA hypomethylation, decreased levels of DNMT1 and DNMT3B, and changes in microRNA profile in all studied cancer cells. Moreover, SFN induced a decrease in m6A RNA methylation pattern that is also considered as an epigenetic regulation at the RNA level, recently discovered. So, SFN may promote genetic instability directly or indirectly by SFN-mediated DNA hypomethylation and/or diminution in m6A RNA methylation pools (Lewinska et al. 2017). Interestingly, in another study, authors demonstrated that SNF upregulates miR-140, which is a negative regulator of cancer stem cell formation in basal-like early stage breast cancer. These results highlight its potential preventive properties for breast cancer (Schnekenburger and Diederich 2015). SNF represents one example of bioactive molecules from

Table 1 Examples of the effect of phytochemicals on DNA methylation in cancer prevention

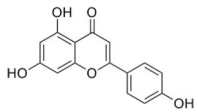
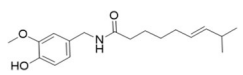
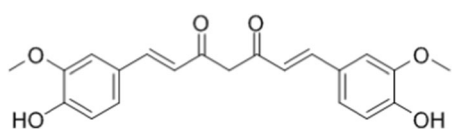
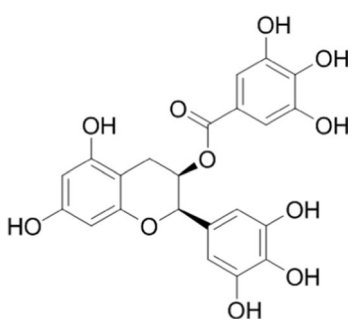
Phytochemical	Structure	Natural sources	Epigenetic modifications	Concentration	Gene targeted	Cancer prevention	References
Apigenin		Parsley, celery, garlic, chamomile tea, Fruits, Chinese herbs	Induces demethylation of gene promotors Reduces expression of DNMTs	1.5–6.5 μM in vitro 20–50 μM in vitro	GST Nrf2 p21 PTEN Cyclin-D1 MAPK PI3-Akt	Increases efficacy of detoxification enzymes; Anti-cancer properties in several malignancies; Inhibits skin tumors development in vivo	Li and Tollefsbol (2010), Shukla and Gupta (2010) and Khan et al. (2018)
Capsaicin		Chilli peppers, *Capsicum genus*	Inhibition of LINE-1 retrotransposition Reactivates HATs expression	30–40 μM in vitro 6–9 μg/ml in vitro	hMOF p21 p53 CDK4 CDK6 cyclin E	Inhibits cell proliferation and induces apoptosis in several cancer cells; Inhibits angiogenesis in vitro and in vivo; could be an carcinogen	Clark and Lee (2016), Wang et al. (2016) and Nishikawa et al. (2018)
Curcumin		Turmenic	Decreases local promoter hyper-methylation Induces global DNA hypo-methylation; Modulates methylation of inflammatory genes; Inhibitor of DNMT1 and DNMT3B, MeCP2	2.5–5.0 μM in vitro 7.5–10 μM in vitro 5–10 μM in vitro 5–10 μM in vitro 100 mg/kg mice—in vivo	COX-2 GST SOD Nrf2 NF-κB RASSF1a RARβ $p15^{INK4B}$ RNAK Neurog1	Reactivates TSGs; Multi-chemoprevention activity; Anti-cancer effect in leukemia, lung, GBM Suppresses colon cancer growth in vivo.	Park et al. (2013), Jiang et al. (2015), Guo et al. (2018) and Khan et al. (2018)
EGCG		Green tea	Modulates demethylation of several TSGs Inhibitor of DNMT1 and DNMT3A; MeCP2	20–50, 100 μM in vitro 50–100 μM in vitro 10 mg/kg mice-in vivo	$p16^{INK4a}$ p21 IL-23 RAR β MGMT hMLH1 hTERT GSTP1 WIF-1	Chemopreventive properties in several cancers; Anti-cancer effect on cancer cell lines; Suppresses tumor growth in colon, prostate and breast cancer mouse models; Promising results in clinical trials for prevention of colon, oral, prostate	Pandey et al. (2010), Singh et al. (2011), Moseley et al. (2013), Schramm (2013) and Khan et al. (2018)

Table 1 continued

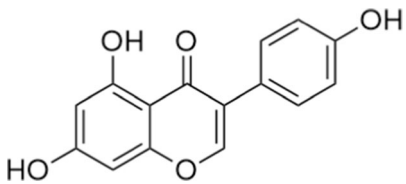
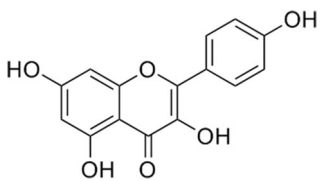
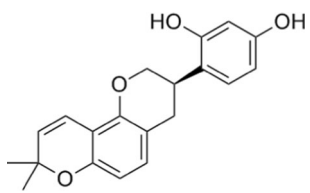
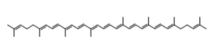
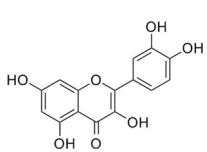
Phytochemical	Structure	Natural sources	Epigenetic modifications	Concentration	Gene targeted	Cancer prevention	References
Genistein		Soybean	Suppresses global DNA methylation; Induces demethylation of TSG promoters; DNMT1 inhibitor	20–40 μM in vitro 20–40 μM in vitro 250 mg/kg mice– in vivo 40–50 μM in vitro	$p16^{INK4a}$ RAR β MGMT hTERT PTEN CYLD	Chemopreventive properties in several cancers; Prevents the development of early colon and gastric neoplasia in vivo; Clinical studies showed breast cancer prevention in Asian women	Fang et al. (2005) and Dietz et al. (2016)
Kaempferol		fruits vegetables	Modulates DNA methylation; Inhibitor of DNMT1, DNMT3B, HDACs	40 μM in vitro 150 mg/kg mice— in vivo 2.5–5 μM 40 μM in vitro	GSTP1 PTEN DACT2	Prevents or reduces bladder and CRC tumorigenesis in vivo; Suppresses cell proliferation and metastasis in vitro in several cancer cell lines	Qiu et al. (2017) and Lu et al. (2018)
Glabridin		Roots of licorice *Glycyrrhiza glabra*	Induces demethylation at promoter of miR-148a and miR-200c; Decreased DNMT1 and DNMT3A expression	15–20 μM in vitro 40–80 μM in vitro	NF-κB AP-1	Anti-tumor effects on several human cancers; Decreases the CSCs-like properties in hepatic and breast cancer in vitro and in vivo	Hsieh et al. (2014), Dietz et al. (2016) and Jiang et al. (2016)
Lycopene		Tomatoes carrots	Induces demethylation of genes promotors Protects global methylation of LINE-1 Inhibits DNMT3A	5–10 μM in vitro 5–10 μM in vitro 40 μM in vitro	GSTP1 ERO1 CLIC-1 NF-κB	Strong anti-oxidant activity Acts in a hormone independent manner in prostate and breast cancers Chemoprevention in several cancers	Fu et al. (2014) and Dietz et al. (2016)
Quercetin		Citrus onion red wine, tea, propolis	Demethylation of gene promoters; Inhibits DNMTs activity	20–50 μM in vitro 75–100 μM in vitro	ER-β $p16^{INK4a}$ RASSF1A AR COX-2	Suppresses the growth of breast, colon, pancreatic ductal adenocarcinoma cells; Prevents carcinogenesis induced by chemicals carcinogens in vivo	Gibellini et al. (2011) and Guo et al. (2015)

Table 1 continued

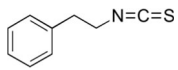
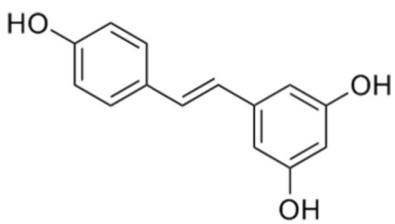
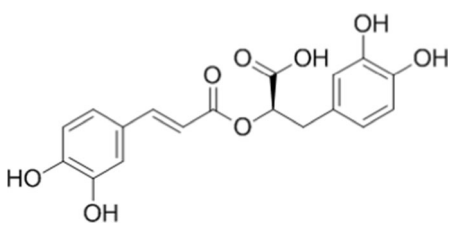
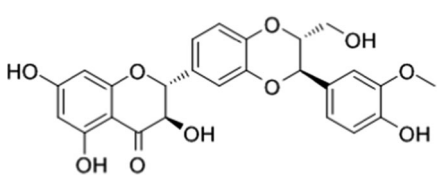
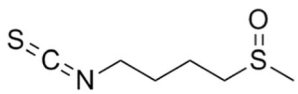
Phytochemical	Structure	Natural sources	Epigenetic modifications	Concentration	Gene targeted	Cancer prevention	References
Phenethyl isothiocyanate (PEITC)		Cruciferous vegetables	Demethylation of several gene promotors; Inhibited DNMTs and HDACs activities	25–50 μM in vitro 2.5–12.5 μM in vitro	CYP1A1 CYP1A2 GSTP1 p21 RASSF1A PcG complex	Strong anti-oxidant activity Multiple chemoprevention effect on several cancers Reduces breast and CRC cancer growth in vivo	Wang et al. (2007), Wang and Chiao (2010), Gupta et al. (2014) and Park et al. (2017)
Resveratrol		Blue berrieCranberries Grapes	Reduced DNA methylation of RASSF1A; Inhibitor of DNMT3B	10–20 μM in vitro 10–20 μM in vitro	COX NF-κB RASSF1A	Multiple anti-cancer effects on several malignant cell lines; Protects against tumor initiation and progression in vitro and in vivo; Reduces breast, colon, liver tumor growth in vivo	Carter et al. (2014), Li et al. (2016a, 2016b) and Khan et al. (2018)
Rosmarinic acid		Rosmarinus officinalis	Inhibitor of DNMT1	20–40 μM in vitro	GSTP1 COX-2 p-ERK IL-6 STAT3	Anti-cancer effect on breast, colon, gastric, prostate, ovarian, skin cancers in vitro In vivo: prevents chemical induced tumor formation on hamster; reduces breast, colon, lung tumor growth in mice	Moore et al. (2016), Venkatachalam et al. (2016) and Khan et al.(2018)
Silibinin		milk thistle (*Silybum marianum*)	Inhibition of DNMTs	50–150 μM in vitro	EGFR $p21^{CIP1}$ $p27^{KIP1}$ NF-κB	Multiple chemopreventive actions in several cancers in vitro and in vivo; Inhibits the chemical induced skin, colon, lung carcinogenesis in vivo	Ramasamy and Agarwal (2008), Ting et al. (2013), Anestopoulos et al. (2016) and Dietz et al. (2016)

Table 1 continued

Phytochemical	Structure	Natural sources	Epigenetic modifications	Concentration	Gene targeted	Cancer prevention	References
Sulforaphane		Cruciferous vegetables, broccoli	Modulates demethylation at gene promotors Global DNA hypo-methylation; Inhibitor of DNMT1, DNMT3B; Modulates miRs expression; Reduces m6A RNA methylation	15–30 μM in vitro 15–30 μM in vitro 1–2.5 μM 10–20 μM in vitro 10–20 μM in vitro 10–20 μM in vitro	Bax GSTP1 COX-2 NF-κB cyclin D2 hTERT Nrf2 NQO1	Prevents growth of breast cancer stem cells in vitro and in vivo; Prevents CRC prostate, skin, oral, pancreatic tumorigenesis in vivo on murine models	Kwon et al. (2007), Hsu et al. (2011), Leone et al. (2017), Lewinska et al. (2017) and Cao et al. 2018

GSTP1 glutathione S-transferase, *DNMTs* DNA methyltransferases, *LINE* long interspersed nuclear element, *Nrf2* nuclear factor (erythroid-derived 2)-like 2, *PTEN* phosphatase and tensin homolog, *MAPK* mitogen-activated protein kinase, *CDK* cyclin-dependant-kinase, *COX2* cyclooxygenase 2, *NQO1* NAD(P)H: quinone oxidoreductase 1, *RASSF1* Ras association domain-containing protein 1, *NF-kB* nuclear factor kappa-light-chain-enhancer of activated B cells, *SOD* superoxide dismutase *RAR-β* retinoic acid receptor beta, *MGMT O6* methylguanine-DNA-methyltransferase, *IL* interleukin, *MutL* homolog 1, *WIF1* Wnt inhibitory factor 1, *DACT2* dishevelled binding antagonist of beta catenin 2, *AP-1* activator protein 1, *ERO1* endoplasmic reticulum oxidoreductin 1, *CLIC-1* chloride intracellular channel 1, *ER-β* estrogen receptor beta, *AR* androgen receptor, *CYP1A1/2* cytochrome P450, family 1, subfamily A, polypeptide ½, *PcG* polycomb group, *p-ERK* phosphorylated Extracellular signal-regulated kinase, *STAT3* signal transducer and activator of transcription 3, *hTERT* human telomerase reverse transcriptase

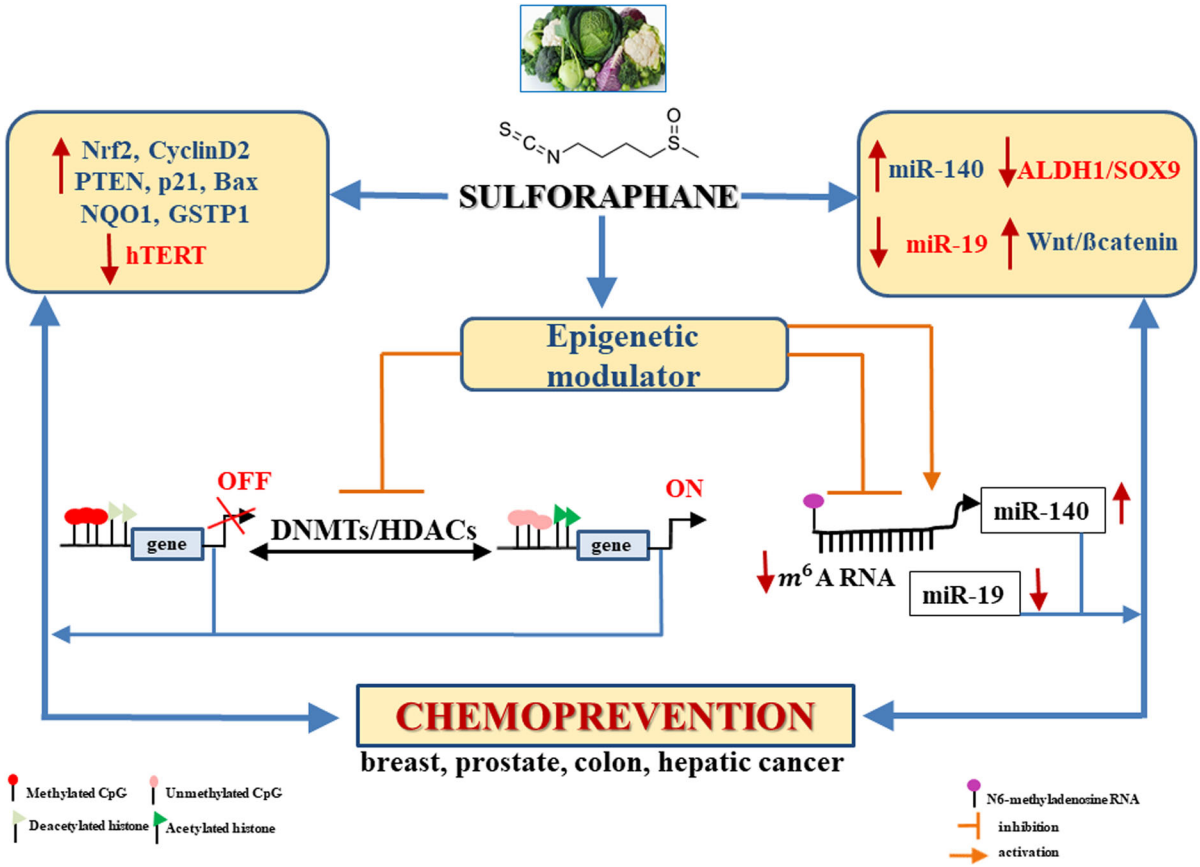


Fig. 3 Sulforaphane modulates epigenetic mechanisms in chemoprevention. Sulforaphane induces activation of Nrf2 gene and upregulates the expression of anti-oxidative enzymes, related with cancer prevention mechanism. Accumulating evidence suggests that the anti-cancer properties of sulforaphane could be at least partially mediated by its effect on epigenetic mechanisms. Sulforaphane is a well-described DNMTs and HDACs inhibitor, reducing gene promoter-specific methylation and increasing total and promoter-specific histone acetylation in cancer cells. Also, SFN can modulate the expression of several microRNAs, and at mRNA level it was associated with decreased 6-adenosine RNA methylation

natural sources that can modulate different epigenetic mechanisms in order to restore the normal function of genes involved in chemoprevention processes, as we show in Fig. 3.

To conclude, evidence-based data from both preclinical and clinical trials are now adding to support the benefits of bioactive compounds in preventing or mitigate tumor growth. From the multiple processes altered in tumor cells, epigenetic ones, namely DNA methylation, can be modulated with the help of phytochemicals commonly found in natural foods and spices. In support of such arguments, SNF holds a strong case, showing new beneficial sides of its well documented anti-oxidant activity. Whether these active compounds will continue to be only treatment adjuvants or will seize the lead in antitumor therapy, remains to be established by large cohort and epidemiologic studies. Substantial experimental evidence indicates the potential importance of dietary and bioactive compounds in cancer prevention, but identifying direct relationships between diet and cancer in observational epidemiological studies and intervention trials had proven challenging. Study design issues, imprecise dietary assessments, and a lack of consideration of tumor heterogeneity generally attenuate relative-risk estimates in observational studies; dietary biomarkers and characterization of etiological subtypes of cancers can help to better identify diet–cancer associations.

Conclusions

Epigenetic changes, such as DNA methylation, can be heritable, but are also influenced throughout life by environmental factors, such as diet, thus providing a novel avenue for lifestyle or therapeutic interventions. Unlike the conventional drugs, phytochemicals have multiple targets and are thus of potential value in diseases like cancer, where multiple pathways are altered. Moreover, they have selective toxicity targeting the cancer cells while showing negligible damage to normal cells. As demonstrated from taxol to sulforaphane, there is an unprecedented potential in exploring the herbal diversity for anti-cancer drug candidates. DNMT inhibitors that are currently used in clinical trials are non-selective cytosine analogues with considerable cytotoxic side effects. Several natural products, such as EGCG, curcumin, sulforaphane, from diverse chemical classes, have shown DNMT inhibitory activity, but this property needs more in-depth investigations.

Pre-clinical and clinical studies addressing the relevance and validity of in vitro experimental outcomes as well as analysis of safety profile, dose, and length of treatment are to be validated by future trials. New technologies and advances in genetics, epigenetics and metabolomics, and consideration of the influence of the microbiome, will expand our understanding of the role of dietary phytochemicals in cancer risk and disease progression.

Whilst it may be impossible to avoid the initiating mutagenesis, cancer could be prevented if cells bearing the initiating lesion(s) could be identified and the faulty epigenetic process corrected with the help of active phytochemicals. The epigenetic modulation of the early cancer events discovered in humans using phytochemicals remains a hope for cancer therapy.

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Compliance with ethical standards

Conflict of interest The authors confirm that there are no conflicts of interest.

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