

Natural compounds as epigenetic modulators: Reversing DNA hypermethylation in cancer (Review)

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Abstract. The development of cancer involves an intricate mechanism, including oncogene activation and tumor suppressor gene inactivation, often driven by epigenetic alterations. Notably, these epigenetic modifications, such as DNA hypermethylation, are dynamic and reversible, and emerging research suggests that dietary factors can influence them. Recent findings have examined the intricate associations between dietary compounds and cancer development, with a particular focus on the mechanisms through which natural components affect epigenetic alterations. Natural compounds have gained significant attention for their potential in chemoprevention and treatment due to their capacity to modulate DNA methylation patterns, particularly at promoter CpG islands. Chemotherapy and radiation therapy are effective cancer treatments and are often associated with severe adverse effects. As a result, there is increasing interest in exploring natural substances as alternative therapeutic options. The present review focuses on natural compounds that can reverse hypermethylation, providing a potentially safer approach for targeted cancer treatment. These bioactive agents, abundant in natural compounds, exhibit the potential to combat cancer by inhibiting metastasis, inducing cell cycle arrest and reversing DNA hypermethylation. The present review aimed to provide further in-depth mechanistic insight into the mechanisms through which natural compounds, such as genistein, resveratrol, quercetin and capsaicin modulate DNA hypermethylation in various types of cancer, including breast, cervical, prostate and neuroblastoma. By combining *in silico*, *in vitro* and *in vivo* approaches, it uniquely integrates computational and

experimental evidence to demonstrate compound-specific reversal of epigenetic marks.

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1. Introduction

Cancer is a devastating disease distinguished by increased cell division, ranking among the leading causes of mortality worldwide. Its development and progression result from a combination of genetic and epigenetic alterations (1). Simultaneous genomic and epigenetic alterations lead to the dysregulation of numerous genes, including the expression or suppression of tumor suppressor genes (2). These modifications are heritable and have the potential for therapeutic intervention. DNA methylation and other epigenetic processes have emerged as a rapidly expanding field of investigation. They govern the critical stages of cell development, differentiation and responses to environmental factors, such as dietary variations, chemical exposure, radiation, hormones and tobacco smoke. DNA methylation involves the addition of a 'methyl' group at the fifth carbon position in cytosine (3). Glucose-regulated protein 78, Src, Toll like receptor 7, caveolin-1 and dopamine receptor D2 are involved in JEV binding and entry into neurons, with these receptors also known for their roles in carcinogenic activities (4). Aberrant modifications in DNA methylation patterns can either initiate cancer or serve as indicators of malignant tumor growth (5). Hypermethylation involves an excessive increase in the methylation of adenine and cytosine residues in DNA. CpG region hypermethylation within tumor suppressor genes causes transcriptional repression and the loss of their tumor-suppressing functions, contributing to carcinogenesis (6).

The hypermethylation of tumor suppressor genes indicates their inactivation during cancer development. A notable example is the frequently hypermethylated CpG

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island found within the ras association domain family 1A promoter region. The development of potent anticancer drugs often occurs through natural compounds, structural alterations of existing natural compounds, or the production of entirely novel molecules. The pursuit of improved cytotoxic agents remains pivotal in the development of modern anticancer drugs. The derivatives of several marine organisms, plants and microorganisms are considered promising starting points for enhancing their remedial efficacy through manipulation at the molecular level, owing to the vast structural diversity and bioactivity potential of natural chemicals (7). These bioactive anticancer compounds can impede cancer cell proliferation, reverse DNA hypermethylation in cancer cells, inhibit metastasis and halt the cell cycle. Specific compounds, including naturally occurring polyphenols, can function as hypermethylation agents, effectively counteracting the epigenetic suppression of tumor suppressor genes. Notably, curcumin has been discovered for its potential to target and regulate the expression of numerous tumor suppressor genes (8). In cancers, such as esophageal, prostate and cervical cancers, genes such as cyclin dependent kinase inhibitor 2A (CDKN2A, also known as p16INK4a), O-6-methylguanine-DNA methyltransferase (MGMT), retinoic acid receptor (RAR) β , PC3 and suppressor of cytokine signaling 1 (SOCS1), which were previously hypermethylated, have been demethylated through the action of natural compounds, such as genistein and capsaicin (9). These compounds demonstrate the potential of natural substances to reverse epigenetic modifications, offering a promising avenue for cancer therapy (9). Given the limitations of current cancer treatments, there is an ultimate need for novel therapeutic approaches. Natural compounds derived from plants, with their diverse chemical structures and biological activities, offer promising potential in cancer therapy. These compounds have demonstrated the ability to reverse DNA hypermethylation, inhibit cancer cell proliferation, and reactivate silenced tumor suppressor genes. Additionally, some natural substances may serve as biomarkers for early cancer diagnosis. The present review focuses on the potential of natural compounds as epigenetic modulators in cancer therapy, particularly their ability to target and reverse DNA hypermethylation. By elucidating the mechanisms underlying the anticancer effects of these molecules, the present review aims to advance the development of more targeted and effective cancer treatments (2).

2. Promoter hypermethylation

DNA methylation is the addition of a methyl group to a DNA segment, which is a critical step in epigenetic regulation. DNA methylation at specific sites typically inhibits the attachment of proteins responsible for gene interpretation. Demethylation, a process used to remove methyl groups, can activate genes (6). DNA methylation usually results in the silencing of genes, whereas demethylation leads to gene activation. In normal cells, DNA methylation is critical for proper cellular function and development. However, cancerous cells exhibit abnormal patterns in DNA methylation. Tumor-suppressor genes often experience a surge in methylation levels, leading to their silencing, whereas oncogenes, which promote tumor growth,

exhibit reduced methylation levels, resulting in their activation and uncontrolled tumor growth. The dysregulation of DNA methylation is pivotal in the development of diseases, such as cancer (10). DNA methyltransferases (DNMTs) are crucial elements in DNA methylation that facilitate the transmission of methyl groups to DNA sequences in a sequence-specific manner. This enzymatic process occurs during DNA replication and involves the recognition and binding of specific DNA sequences by DNMTs. DNMTs add methyl groups, often from S-adenosylmethionine, to cytosine residues, profoundly affecting gene expression regulation and cellular function (11).

Hypermethylation promotes transcriptional repression in the promoter region in CpG islands of tumor-suppressor genes, thus silencing the gene, which is a hallmark of cancer. CpG island hypermethylation is associated with numerous tumor types and disrupts vital biological processes, including cell cycle control, DNA repair, apoptosis and detoxification. Various hematological disorders, including leukemia, are associated with hypermethylation. Numerous genes, such as estrogen receptor, syndecan 4, multidrug resistance 1, calcitonin, cyclin dependent kinase inhibitor 2B, p21Cip1/Waf1 and others, exhibit hypermethylation in these malignancies. The hypermethylation of CASP8, TMS1 and DAPK genes was observed in childhood acute lymphoblastic leukemia, exhibiting significant differences compared to healthy controls (12). In lung cancer, numerous genes display altered DNA methylation patterns, with RAR β , Ras association domain family member 1A (*RASSF1A*), cyclin dependent kinase inhibitor 2A and *APC* being frequently hypermethylated (11). Dysregulated DNA methylation, either hypomethylation or hypermethylation, disrupts the normal functioning of genes and can lead to the development of various disorders, including cancer. Hypermethylation, which is observed in a number of types of cancer, inhibits tumor suppressor genes, contributing to tumor formation and development. The promoter hypermethylation of tumor suppressor genes, such as p14, p15, p16, p21, p27, p57, p53, p73, RAR β 2, fragile histidine triad diadenosine triphosphatase, death associated protein kinase 1 (*DAPK*), *STAT1* and *RBI* has been detected in paired biopsy and serum samples from cervical cancer patients (13). Abnormal DNA methylation can result in the development of various disorders, including cancer. While there is a general decrease in global CpG methylation, occasional events activate previously repressed oncogenes. Conversely, CGI hypermethylation in gene promoters is a significant marker of various types of cancer. One of the prompt examples of this epigenetic silencing phenomenon was the identification of hypermethylation in the promoter of retinoblastoma tumor suppressor gene in individuals with retinoblastoma (11).

3. Reversal of DNA hypermethylation in cancer cells

The process of the demethylation or reversal of methylation removes methyl groups and allows the gene that has been switched off to resume normal functioning. Research has provided evidence that the dynamic process of reversible CpG island methylation, mediated by DNA methyltransferases, exerts a regulatory influence on the activity of important genes and transcription factors that participate in the modulation of cell progression (2) (Fig. 1).

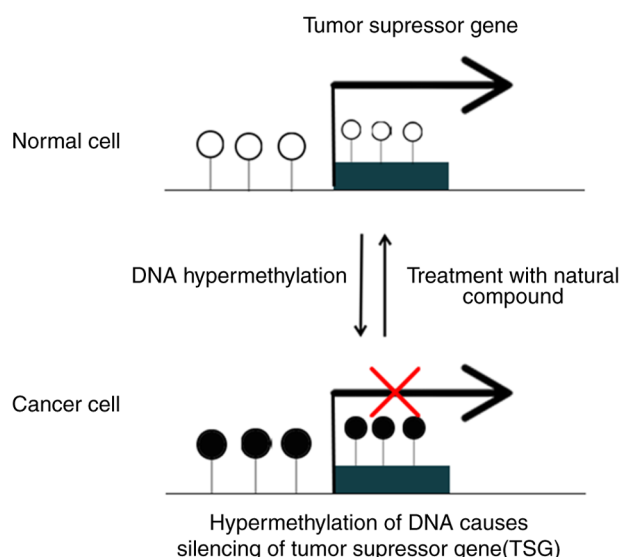


Figure 1. Natural compounds induce demethylation in cancerous cells, which in turn re-activates tumor suppressor genes.

4. Reversal of hypermethylation by natural compounds

In the pursuit of an effective cancer treatment strategy, it is imperative to devise pharmaceutical agents capable of targeting a spectrum of cellular processes encompassing gene expression, signaling pathways, cellular proliferation, intercellular associations and the extracellular matrix. An essential consideration in the selection of these compounds is the imperative need to ensure their safety profile, particularly with regard to undesirable side effects such as cytotoxicity (9). Natural compounds have emerged as a compelling alternative in this context due to their multifaceted attributes. They can induce apoptosis, stimulate tumor suppressor genes by resisting DNA methylation modifications, regulate the cell cycle, activate cell survival proteins and modulate epigenetic mechanisms. Notably, natural compounds have demonstrated the capability to rectify aberrant epigenetic changes, such as DNA hypermethylation, either by reversing hypermethylation or by attenuating the activity of methyltransferases (2). The reversing ability of epigenetic alteration has catalyzed the development of a class of therapeutic agents specifically designed to target epigenetic processes. These agents, known as epigenetic therapies, are engineered to obstruct the activation of genes involved in the initiation of hypermethylation in several cancers (14). The key epigenetic mechanism with the pivotal role of natural compounds, such as genistein, resveratrol, capsaicin, quercetin and epigallocatechin gallate (EGCG) targets the hallmarks of cancer, DNA methylation and the restraining of tumor suppressor genes to reverse the unusual expression of these changes and restore the normal methylation patterns (14) (Fig. 2 and Table I).

Genistein. Genistein is a significant nutraceutical compound that is naturally present in soybean seeds and serves as a precursor to phytoalexins found in legumes. Extensive research has uncovered numerous potential health benefits associated with dietary genistein (15). Moderate exposure to genistein exerts inhibitory effects on several types of cancer,

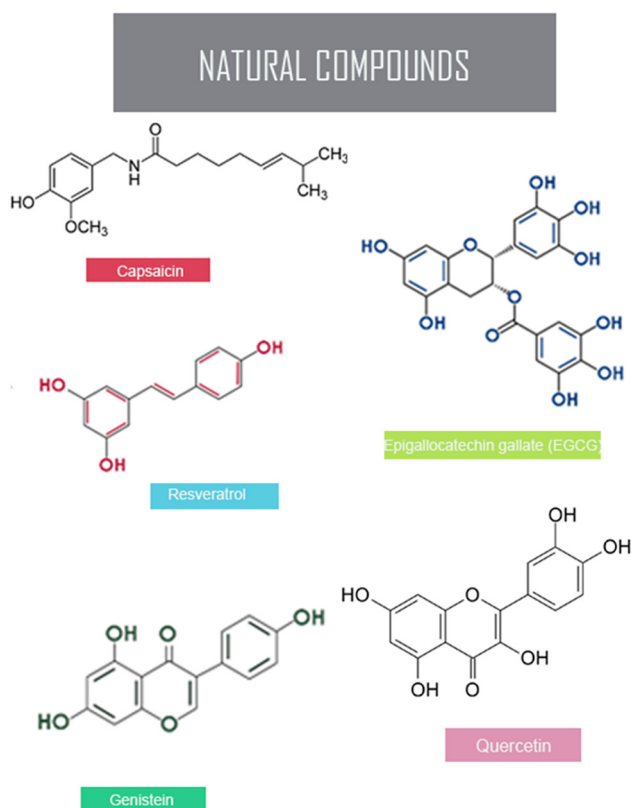


Figure 2. Structural outline of the natural compounds exhibiting properties of demethylation.

such as cervical, colon, esophageal and prostate cancers. The process through which genistein achieves these effects involves a reduction in the activity of DNA methyltransferase, consequently leading to the reversal of DNA hypermethylation (2), as illustrated in Fig. 3. This reversal process leads to gene reactivation that was initially suppressed due to methylation, effectively counteracting epigenetic modifications, specifically DNA hypermethylation (2). In the esophageal cancer cell line, KYSE 510, genistein reactivates previously suppressed genes, such as *p16INK4a* [which is a cyclin-dependent kinase inhibitor that controls the cell cycle by preventing retinoblastoma (Rb) protein phosphorylation, thus blocking progression from the G1 to the S phase], *MGMT* (a DNA repair enzyme that removes alkyl groups from the guanine O6 site, preventing replication errors and protecting the genome from mutations) and *RAR β* (controls the expression of genes involved in cell differentiation, apoptosis, and the suppression of cell growth) (16). This reactivation is attributed to the suppression of DNMT activity by genistein, thereby causing the reversal of DNA hypermethylation followed by the reactivation of methylation-silenced genes. Similar effects were examined in prostate cancer PC3 and LNCaP cells, where it altered DNA hypermethylation and activated the *RAR β* gene (17).

Furthermore, in prostate cancer, specifically in the LNCaP and PC3 cell lines, genistein treatment was proven to transcriptionally reverse the expression of B-cell translocation gene 3 (*BTG3*). These cell lines exhibited complete methylation in the gene promoter region. Genistein treatment led to an increase in *BTG3* mRNA expression and the reactivation of

Table I. Natural compounds demonstrated to have the ability to repair the overexpression of epigenetic modifications, such as DNA methylation, by reversing hypermethylation or by lowering the activity of methyl transferase.

Compounds	Genes	Cell lines/cancer type	(Refs.)
Genistein	<i>p16INK4a</i> , <i>MGMT</i> and <i>RARβ</i>	KYSE 510, PC3 and LNCaP	(16)
	<i>BTG3</i>	LNCaP and PC3 (prostate cancer)	(17)
	<i>GSTP1</i> , <i>EPHB2</i> , and <i>BRCA1</i>	PC-3 and DU-145 (prostate cancer)	(18)
	<i>APC</i> , <i>ATM</i> and <i>PTEN</i>	MDA-MB-231 and MCF-7 (breast cancer)	(21)
	<i>MGMT</i> , <i>RAR</i> , <i>E-cadherin</i>	HeLa cells (cervical cancer)	(22)
	<i>DAPK1</i> and <i>p21</i>		
Capsaicin	<i>SOCS1</i> and <i>CADM1</i>	HeLa (cervical cancer)	(27)
Epigallocatechin gallate (EGCG)	<i>p16INK4a</i> , <i>RARβ</i> , <i>MGMT</i> and <i>hMLH1</i>	KYSE510 and PC3 (esophageal cancer)	(30)
	<i>p16INK4a</i>	A431 (epidermal cancer)	
	<i>p16INK4a</i>	HeLa (cervical cancer)	(31)
	<i>RAR</i> , <i>CDH1</i> and <i>DAPK1</i>	KYSE510 (esophageal cancer)	(29)
	<i>RAR</i> , <i>p16</i> , <i>hMLH1</i> and <i>MGMT</i>	CAL-27 (oral squamous cell cancer)	(29)
	<i>RECK</i>		(32)
Resveratrol	<i>PTEN</i> and <i>RARβ2</i>	MCF-7 (breast cancer)	(36)
	<i>BRCA-1</i>	MCF-7 (breast cancer)	(37)
	<i>RUNX3</i>	B16F10 (malignant melanoma)	(39)
Quercetin	<i>RASSF1A</i>	HeLa (cervical cancer)	(41,44)
	<i>p16INK4a</i> , <i>CDH1</i> , and <i>IGFBP7</i>	RKO cell lines (colorectal cancer)	(42)
	<i>MGMT</i> , <i>GSTP1</i> , <i>APC</i> , <i>SOC51</i> , <i>CDH1</i>	HeLa (cervical cancer)	(45)

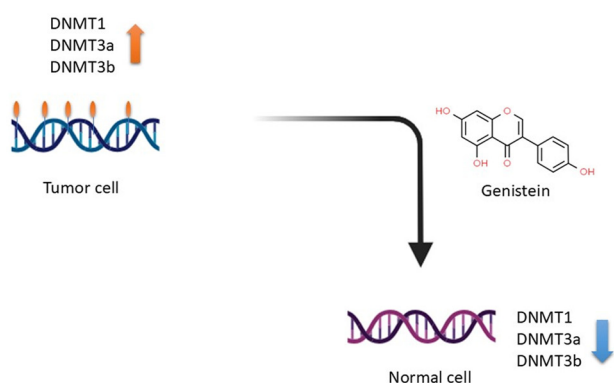


Figure 3. Schematic illustration of the action of genistein on tumor cell in the reversal of the hypermethylation of tumor suppressor genes.

the *BTG3* gene by reducing promoter methylation, primarily through the inhibition of DNA methyltransferase activity, thus modulating the hypermethylation pattern (17). The demethylating ability of genistein has been demonstrated on the promoters of the glutathione S-transferase Pi 1 (*GSTP1*), EPH receptor B2 (*EPHB2*) and breast cancer susceptibility (*BRCA1*) genes in PC-3 and DU-145 prostate cancer cell lines. *BRCA1* and *BRCA2* are cellular proteins involved in DNA repair. They are normally expressed in the breast, ovaries, prostate and other tissues. Their germline mutation is the cause of hereditary breast-ovarian cancer syndromes. Prostate cancer cells proliferate when *EPHB2* is silenced. *EPHB2* expression is decreased in prostate cancer tissues and

fully inactivated in the metastatic prostate cancer cell line, DU145, suggesting a link to disease progression (18). While *GSTP1* protects prostate epithelial cells from DNA damage caused by carcinogens and oxidative stress, its absence may leave prostate cells vulnerable to genomic inserts. Using the Methyl Profiler DNA methylation assay, the methylation levels of the prostate cancer cell lines were previously quantified. It was demonstrated that the methylation pattern of *GSTP1*, *BRCA1* and *EPHB2* promoters was decreased by genistein. Therefore, genistein may be a preventive agent against prostate cancer (18).

Based on research studies on neuroblastoma, extensive promoter hypermethylation has been observed in the *p53* and *CHD5* genes. Genetic therapy regulates the activity of *CHD5* and *p53* and reduces *CHD5* hypermethylation levels. Genistein also functions as a DNMT inhibitor, which considerably lowers DNMT3b expression. Genistein functions in tandem with *p53* to restrict neuroblastoma growth, which is potentially mediated by the WNT pathway, by lowering the methylation of the *CHD5* promoter and increasing *CHD5* and *p53* expression levels (19). In a study on the development of breast tumors, the administration of genistein to SHR cells inhibited cell growth in pre-cancerous cell populations. Notably, this result has been linked with an upregulation in the activation of crucial tumor suppressor genes, specifically p16 and p21, while concurrently with the downregulation of tumor-promoting gene expression, namely *c-MYC* and *BM11*. These genes, previously silenced due to aberrant epigenetic modifications, are well-established contributors to cell cycle arrest, exerting growth-inhibitory effects across various cancer cell lines (20).

Genistein has been proven to reduce DNA methylation in the tumor suppressor genes of the breast cancer MDA-MB-231 and MCF-7 cell lines. The administration of genistein reactivated methylation-silenced tumor suppressor genes, such as *APC*, *ATM* and *PTEN* by direct contact with the DNMT1 enzymatic domain and suppressed DNMT1 activity in cancer cells (21). In a previous study, it was shown that genistein suppressed DNMT activity, induced demethylation and activate methylation-silenced genes. The treatment of KYSE 510 cells with genistein caused the reversal of DNA hypermethylation and reactivation of the *RAR β* , *p16INK4a* and *MGMT* genes (16). Studies on DNMTs *in vitro* and *in silico* have been conducted to investigate the function of DNMTs on human cervical cancer HeLa cells following treatment with genistein. The effects of genistein treatment on tumor suppressor genes, such as *MGMT*, *RAR*, *E-cadherin* *DAPK1* and *p21* and the methylation pattern of their promoter regions were observed. Genistein treatment reduced the enzymatic activity of DNMTs. The promoter region methylated DNA was reversed, and gene expression was restored following time-dependent exposure to genistein (22). Prolonged therapy with genistein increased expression in the MDA-MB-468 breast cancer cell line and led to the demethylation of the promoter region. Furthermore, the *RAR β 2* cistron in MCF-10A cells was demethylated by genistein. The ability of genistein to demethylate hypermethylated genes in MCF-10A, MDA-MB-468 and MCF-7 breast cancer cells was further examined in these studies, although a negligible effect was shown in the *GSTP1* gene in MDA-MB-468 and MCF-7 breast cancer cells (21).

Genistein has been demonstrated to function as an epigenetic modulator in a neuroblastoma model by inhibiting tumor development and the methylation state of the CDH5 promoter region. DNA methyltransferase activity was inhibited *in vivo* as a result of the downregulation of DNMT3 (19).

In general, natural compounds have been shown to be able to reverse hypermethylation and the reactivation of tumor suppressor genes (23).

Capsaicin. Red chili peppers contain a large amount of the bioactive chemical capsaicin. In addition to its antifungal and anti-inflammatory properties, capsaicin has anticancer properties. The chemopreventive properties of capsaicin are extensive. Capsaicin also affects the activation of genes involved in cancer cell survival, the development of new blood vessels, metastasis and the inhibition of cancer cell development by altering the activity of enzymes involved in DNA methylation, thereby causing the reversal of methylation. Capsaicin alters DNA expression by DNMTs. By inhibiting their activity, capsaicin prevents DNA methylation, thereby promoting a more standard epigenetic profile (24). Scientific research has shed significant light on the medicinal potential of capsaicin in treating the suppression of *SOCS1* and cell adhesion molecule 1 (*CADM1*) in human papillomavirus cervical cancer cell line expression caused by hypermethylation. Additional investigations are warranted to explore the fundamental mechanisms and validate the clinical significance of capsaicin as a specific intervention for modifying DNA methylation and expression patterns of genes associated with medical conditions (25). Capsaicin treatment affects the *Par-4* gene and exhibits the ability to lower the

levels of DNA methylation in the prostrate cancer cell line *PC-3* gene. The process of reversing hypermethylation holds significant potential for reactivating tumor suppressor genes that were initially suppressed. As a result, the development of cancer cells can be effectively suppressed (26).

In a previous *in silico* study, tumor suppressor in lung cancer 1 (*TSLC1*), which has excessive methylation, became less active and lost its function, leading to the development of cervical tumors. *TSLC1* encodes a cell adhesion molecule essential for cell-cell interactions and regulating proliferation. In cervical cancer, *TSLC1* is often inactivated by promoter hypermethylation or deletions, disrupting cell adhesion and leading to increased proliferation, invasion, and metastasis. This inactivation contributes to tumor progression and the aggressive nature of the disease. Capsaicin was tested with *TSLC1* and exhibited a strong binding connection. Detailed analyses revealed that capsaicin forms bonds with *TSLC1* in specific ways. Considering this computer-based study, capsaicin also showed promising results as a potential option to reactivate suppressed *TSLC1* due to excessive methylation. This could have implications for the treatment of cervical cancer (27). The DNA methylation of *SOCS1* and *CADM1* was previously examined using methylation-specific PCR on HeLa cells from cervical adenocarcinoma. *CADM1*, a cell adhesion molecule vital for tissue architecture, is downregulated in cervical adenocarcinoma due to promoter hypermethylation. This disruption in cell adhesion promotes tumor cell detachment, invasion and metastasis, while *SOCS1* encodes a protein that regulates cytokine signaling to control cell growth and immune responses. In cervical adenocarcinoma, its inactivation by promoter hypermethylation leads to uncontrolled cytokine signaling, promoting tumor progression through abnormal cell proliferation and immune evasion. The cells were treated with capsaicin followed by 72 h of incubation and treatment was repeated for a further 6 days. The findings of *in vitro* analyses revealed that hypermethylation plays a significant role in suppressing *CADM1* and *SOCS1* expression. Capsaicin causes the reversal of hypermethylation in *CADM1* and *SOCS1* expression (25). The effectiveness of capsaicin in restoring the expression of *CADM1* and *SOCS1* was further supported by the evident changes observed in methylation-specific and unmethylation-specific patterns during methylation-specific PCR. These alterations indicated a notable effect on the pattern of methylation associated with the regulatory regions of *CADM1* and *SOCS1*. The observed changes in the patterns have proven that capsaicin treatment affected the methylation status, triggering the restoration of *CADM1* and *SOCS1* expression (25).

EGCG. EGCG is a potent bioactive compound belonging to the family of catechins found in green tea. Its notable antioxidant, anti-inflammatory and potential anticancer properties have attracted considerable interest in recent times, particularly in the context of chemoprevention and epigenetic modifications, such as the methylation of DNA (2). Epigenetic alterations such as hypermethylation are involved in the growth of various types of cancers. Previous findings have shown that EGCG can repair hypermethylation, providing a viable route for cancer therapeutics based on epigenetics (28). The capacity of

EGCG in chemoprevention and its marked ability to mitigate hypermethylation, demonstrate its prospects as an innovative therapeutic agent (28). The administration of EGCG to the KYSE 510 esophageal cancer cell line, for durations spanning from 12 to 144 h, resulted in the hypermethylation of *p16INK4a*, *RAR β* , *MGMT* and *hMLH1* to be reversed in a manner that was dependent on time and concentration (29). It was previously reported that in KYSE 150 esophageal cancer cells, PC-3 cells and HT-29 colon cells, EGCG demethylated the CpG regions and reactivated methylation suppressed genes, such as *RAR β* , *MGMT*, *p16INK4a*, glutathione S-transferase and human mutL homologue 1 (29).

Another study demonstrated that in epidermal carcinoma A431 cells, the tumor suppressor gene *p16INK4a* was reactivated after being silenced. EGCG administration reduced the levels of methylation, DNMT activity and protein levels of DNMT1, DNMT3a and DNMT3b in the A431 cancer cell line (30). It has also been demonstrated that EGCG modifies epigenetic changes in HeLa cells. The interaction between EGCG, DNMT3B, promoter methylation, and *RAR*, *CDH1* and *DAPK1* expression in EGCG-treated HeLa cells was previously examined using molecular modeling. The treatment of HeLa cells with EGCG reduced DNMT activity. Molecular modeling results established that EGCG suppressed DNMT3B activity. The restoration of tumor suppressor genes in HeLa cells was caused by significant alterations in the pattern of methylation of these genes induced by EGCG (31). It was previously demonstrated that EGCG treatment reactivated the *hMLH1*, *p16INK4a*, *RAR* and *MGMT* genes in HT-29 and KYSE 150 cells (29). It was also discovered that EGCG administration reactivated the methylated silenced genes, *RAR*, *p16*, *hMLH1* and *MGMT* in the KYSE 510 esophageal cancer cell line. EGCG treatment resulted in the activation of epigenetically silenced genes (29).

In a previous study, it was observed that in the oral squamous cell carcinoma cell line, CAL-27, EGCG treatment caused the partial hypermethylation of the *RECK* gene. *RECK* is a tumor suppressor that controls matrix metalloproteinases (MMPs), crucial for extracellular matrix degradation. In oral squamous cell carcinoma, *RECK* is often downregulated due to promoter hypermethylation or genetic changes, leading to an increased MMP activity, which enhances tumor invasion and metastasis (32). Reduced *RECK* levels are associated with more aggressive tumors and a worse prognosis. EGCG reverses the process of hypermethylation and activates the *RECK* gene, restraining oral squamous cell cancer invasiveness and translocation. EGCG was found to decrease the DNA methylation of the *RECK* gene in the cell lines (32). In another study, quantitative PCR and methylation-specific PCR were employed to assess the expression of the *SOCS1* promoter methylation levels. EGCG treatment induced an increase in *SOCS1* expression and the promoter demethylation of *SOCS1* was also upregulated (33). A previous study also proved that EGCG affected DNA hypermethylation and head and neck squamous cell carcinoma (HNSCC) development. EGCG treatment decreased the broad DNA methylation levels in HNSCC cells (SCC-1 and FaDu). Following the administration of EGCG for a significant amount of time, DNA hypermethylation was effectively reduced up to 70-80%. The transformation of

5-methylcytosine into 5-hydroxymethylcytosine in HNSCC cells provided evidence that DNA hypermethylation was suppressed in these cells. Treatment with EGCG markedly decreased DNMT activity in HNSCC cells, decreasing it to 80% in FaDu and 60% in SCC-1 cells (34). In another study, in the Apc(Min/+) mouse model of colorectal carcinogenesis, EGCG treatment reduced tumor formation and reversed RXR α gene silencing. EGCG decreased CpG methylation in the RXR α promoter, restoring its expression and highlighting the role of EGCG in epigenetically inhibiting tumorigenesis (35).

Resveratrol. Resveratrol is a distinct polyphenolic compound obtained from peanuts or grapes. The broad potential of resveratrol includes the ability to fight various tumors, cell development, cell death and angiogenesis. It has gained the interest of researchers involved in the exploration of chemopreventive remedies. In a scientific review focusing on the non-invasive MCF-7 breast cancer cell line, it was demonstrated that treatment with resveratrol induced the reversal of hypermethylation and the re-activation of the tumor suppressor genes, *PTEN* and *RAR β 2*, and a concurrent reduction in the activity of DNMT1, and an elevation in the levels of *p21* (36). It has been reported that resveratrol alters DNMT1 activity and the methyl binding domain protein-2 with the promoter of the *BRCA-1* gene to prevent the tumor suppressor *BRCA-1* from being epigenetically silenced in MCF-7 breast cancer cells (37). Research has shown that resveratrol demethylates *RASSF1A* in females with a higher risk of developing breast cancer. In a previous study, individuals with an elevated risk of developing breast cancer were treated with resveratrol for 2 weeks. Mammary ductoscopy samples were used to examine the methylation of cancer-associated genes, including *RASSF1A*. An increase in trans-resveratrol levels reduced *RASSF1A* methylation (10). It has been reported that resveratrol causes the reversal of promoter hypermethylation and the restoration of tumor suppressor genes. Resveratrol therapy appears to reduce the activity of DNMT1/3b and activate *RASSF-1* in breast cancer cell lines (38). Resveratrol interacts with hypermethylation in the *RUNX3* promoter gene. Researchers have used MSP to assess methylation following treatment, and the results have revealed the marked methylation in the *RUNX3* promoter of B16F10 cells of malignant melanoma, coupled with faint unmethylated segments. Resveratrol doses for a >48 h gradually attenuated *RUNX3* promoter methylation and increased unmethylated areas. Resveratrol exhibited a potent ability to induce significant dose-dependent demethylation (39). In experimental models of mammary carcinogenesis using ACI rats, trans-resveratrol has demonstrated significant antineoplastic properties, primarily through the downregulation of DNMT3 expression (40).

Quercetin. Quercetin is a potent natural antioxidant flavanol that may be derived from a broad category of dietary sources, such as apples, grains, onions, tea, red wine, citrus fruit, and a number of other types of leaves (2). The anticancer effects of quercetin are associated with altering mitogenic signaling, cancer cell metastatic, angiogenesis, the cell cycle and apoptotic signaling pathways, as well as by reversing

epigenetic alterations, such as DNA hypermethylation (41). It was previously shown that quercetin modified the pattern of RASSF1A methylation in the HeLa neoplastic cell line. The administration of quercetin for 6 days resulted in the reverse hypermethylation of the *RASSF1A* gene. The research outcomes revealed that the administration of quercetin to cervical cells effectively altered the hypermethylation level of RASSF1A, as documented by Lugli *et al* (42). Another study was performed to demonstrate the significance of quercetin at the promoter methylation region of several genes that were silenced due to DNA hypermethylation in colorectal cancer RKO cell lines. The genes *p16INK4a*, *CDH1* and *IGFBP7* were examined. These results suggest that quercetin can reverse the methylated-promoter phenotype in the RKO colorectal cancer cell line (43).

In vivo and *in vitro* studies have shown that quercetin may function as a preventive measure against colon cancer (2). The MTT assay has been used in *in vitro* studies and has revealed the capacity of quercetin to prevent the development of the colon cancer cell line, RKO. The hypermethylation of *p16INK4a* was successfully reversed after quercetin therapy for 120 h. The expression of *p16INK4a* was restored in a concentration-dependent manner. This demonstrates that quercetin exerts its chemopreventive properties by demethylating the *p16INK4a* gene promoter (2). Quercetin has the potential to inhibit DNMTs in DU145 and PC3 prostate cancer (CaP) cell lines. The hypermethylation of the androgen receptor (AR) gene promoter is linked to CaP defiant to androgen-deprivation therapy with antiandrogens. In AR-negative cell lines, quercetin and curcumin restored AR mRNA and protein concentrations through global hypomethylation, which led to the initiation of apoptosis by mitochondrial depolarization (44). It has been proven that quercetin can alter the methylation levels of RASSF1A in the HeLa cell line. The hypermethylation of RASSF1A was reversed after 6 days of treatment with quercetin. Quercetin was administered at different doses for different periods of time to examine the reversal of hypermethylation in the *RASSF1A* gene. The results demonstrated that the *RASSF1A* gene in HeLa cells was demethylated after 6 days of therapy (14).

It has been proven that quercetin strongly affects the activity of DNMTs. In a previous study, nuclear extracts were observed to suppress the activity of the DNMTs by 32 and 49%, respectively, when quercetin was added to the mixture in contrast to that of the untreated extract, and it was discovered that the broad methylation levels of the HeLa cell line were decreased. Almost 50% of the methylation was reduced by quercetin treatment within 24 h, and further administration for 24 and 48 h decreased the methylation of DNA to 15 and 36% of the control, respectively. The methylation levels of the TSGs to *DAPK1* (7%), *RASSF1* (9%), *VHL* (10%), *PTEN* (11%), *RARB* (19%), *MGMT* (22%), *GSTP1* (24%), *APC* (31%), *SOC51* (58%) and *CDH1* (60%) were all attenuated by quercetin treatment (45). Another study was conducted to examine the impact of quercetin on cellular immunity against cancer via the IL15 gene. The cell lines A549 and HeLa were grown *in vitro* and exposed to quercetin at various doses. RT-qPCR was performed to examine the transcription activity of IL15 and DNA

methyltransferase. Treatment with quercetin in HeLa and A549 cells reduced the expression Quercetin reduced the production of IL15 by increasing the methylation of the IL15 promoter, thus inhibiting the expansion of malignant cells (46). In xenograft models of MCF-7 (breast cancer) and CT-26 (colon cancer), quercetin administration at various doses (50, 100, and 200 mg/kg) resulted in a notable reduction in tumor volume. This decrease was observed as early as the 18th day in CT-26 tumors and the 20th day in MCF-7 tumors post-treatment, demonstrating quercetin antitumor potential through epigenetic regulation mechanisms (47).

5. Conclusion and future perspectives

It can be concluded that bioactive compounds of natural origin can serve as inhibitors of DNMT activity and mitigate promoter hypermethylation. In addition, the demonstrated the capability to facilitate the re-expression of genes previously subjected to methylation-induced silencing, thereby orchestrating the reversal of epigenetic alterations implicated in oncogenesis. Compounds such as genistein, quercetin, resveratrol, capsaicin and EGCG can reverse the hypermethylation of various genes that cause cancer. Further research into natural compounds as therapeutic epigenetic agents is essential to fully explore their potential in cancer treatment. While some compounds have yielded promising results, further research is required in order to fully elucidate their mechanisms of action and effectiveness. By expanding studies to include a wider range of natural substances, novel, potent epigenetic agents can be identified that may offer novel approaches to cancer therapy. Research on natural compounds could lead to the development of more targeted, less toxic treatments, ultimately improving patient outcomes and advancing the field of oncology.

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Authors' contributions

F conducted a significant portion of the literature search and drafted substantial parts of the manuscript. KSS contributed to editing specific sections of the manuscript. RM edited and proofread the manuscript. AKJ contributed to the initial idea and scope of the review, provided critical review and feedback on the manuscript, and approved the final version of the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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